

**On the
Pharmacological Control of
Mucociliary Activity in the
Rabbit Maxillary Sinus**

in vivo

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IN THE RABBIT MAXILLARY SINUS IN VIVO

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The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals

- I. Hybbinette, J.-C. & Mercke, U.
A method for evaluating the effect of pharmacological substances on mucociliary activity in vivo.
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- II. Hybbinette, J.-C. & Mercke, U.
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- III. Hybbinette, J.-C. & Mercke, U.
Effects of sympathomimetic agonists and antagonists on mucociliary activity.
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- IV. Hybbinette, J.-C.
A pharmacological evaluation of the short-term effect of cigarette smoke on mucociliary activity.
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INTRODUCTION

It has long been held that the mucociliary (m.c.) apparatus of the airways serves to remove inhaled particles from the epithelium lining the airways, thereby contributing to host defence. Symptoms related to the respiratory tract are among the most frequent reasons why patients consult their physicians, and various pharmacological substances are prescribed for the treatment. Information about the therapeutical benefits or possible adverse effects of such drugs on the m.c. activity of the airways is, however, sparse and often conflicting. The effect of cigarette smoke on m.c. activity is also of clinical interest since smoking is believed to play an important role in the pathogenesis of airway diseases.

Several drugs commonly used in clinical practice act on the autonomic nervous system. In addition nicotine in cigarette smoke can also be expected to influence the autonomous control of the mucous membranes (Keal, 1977; Wanner, 1977; Parke, 1978).

The role of the sympathetic and parasympathetic nervous system in the control of m.c. activity has been investigated for decades, but several questions still remain unsolved and reported data are often contradictory. The inaccessibility of the m.c. apparatus under normal, physiological conditions has made it difficult to evaluate the effects of drugs on the m.c. activity.

Thus it is of both clinical and pharmacological interest to develop a test model which will enable m.c. activity to be recorded under as physiologically natural conditions as possible, and with its aid to study the influences on the m.c. apparatus of cigarette smoke and of drugs acting on the autonomic nervous system.

A. Structure and function of the mucociliary apparatus of the airways

In the mammalian airways a ciliated mucous membrane covers the nose, paranasal sinuses, nasopharynx, auditory tubes and middle ears, and the lower airways from the larynx to the end of the terminal bronchioles.

The mucous membrane consists of a ciliated epithelium resting on a basal membrane and an elastic lamina propria mucosae. The submucosa is made up of loose connective tissue containing fat cells, blood vessels, nerves and submucosal glands (Jeffery & Reid, 1977).

The epithelium of the mucous membrane is a ciliated, pseudostratified columnar epithelium covered by a blanket of mucus. Its ultrastructure has been reviewed by among others Messerklinger (1966), Rhodin (1966), Alexander et al. (1975), Jeffery & Reid (1975), Breeze et al. (1976), Vidic & Tandler (1976), Jeffery & Reid (1977), Jeffery (1978) and Reid & Jones (1979). The present description is mainly based on their works.

Several distinct epithelial and mesenchymal cell types have been recognized in the epithelium (Jeffery, 1978). However, variations exist regarding occurrence depending on species and location examined (Jeffery, 1978).

The ultrastructure of the rabbit maxillary sinus mucous membrane (the animal and organ studied in the investigations reported in this thesis) does not seem to have been studied before. Five different cell types have been identified in the rabbit tracheobronchial epithelium: ciliated, mucous (lucent granules), Clara, basal and Kultschitzky cells (Jeffery, 1978).

Of these cell types mentioned two are secretory: mucous and Clara cells. The now obsolete term "goblet cell" refers to a cell distended with secretory

granules. This term excludes a cell with granules of the same type but fewer in number. For this reason the term "mucous cell" is preferred for that secretory cell (see Reid & Jones, 1979). The secretory properties of the epithelium will be discussed below in section B.

In the mammalian respiratory tract ciliated and secretory cells make up the bulk of the respiratory epithelium, and are the only cell types that directly contribute to m.c. function (Andersen, 1971). The ciliated cells are more numerous than any other cell type and in the human tracheobronchial tract there are approximately 5 ciliated cells for every one secretory cell. The ultrastructure of the ciliated cell is almost identical in different airway regions and also in different species (Engström, 1951; Fawcett & Porter, 1954; Iravani, 1968; Sleight, 1971; Afzelius, 1979; Reid & Jones, 1979). The occurrence of cilia in man and in the rabbit is believed to be similar (Negus, 1965).

The mucus produced by epithelial secretory cells is augmented by secretion from submucosal glands embedded in the airway wall. The structure, distribution and histochemical characteristics of these glands in the respiratory airways have been reviewed by among others Meyrick et al. (1969), Spicer et al. (1973) and Vidic & Tandler (1976). It is suggested that the frequency of these glands in different mammals is variable and does not always resemble that of man (Haller, 1969). In the rabbit tracheobronchial airways only few such glands have been identified (Haller, 1969), whereas they are numerous in the rabbit maxillary sinus (Hybbinette & Uddman, unpublished data).

The cilia on the ciliated cells beat in one plane with a fast effective stroke and a slower recovery stroke. This pattern of ciliary motion is remarkably uniform across different species (Wilson et al., 1975). The motion of adjacent cilia is coordinated (metachronism)

creating least interference between adjacent cilia (Gray, 1922; Sleight, 1966). In the airways, the effective stroke of ciliary motion is directed towards the pharynx, whereas the metachronical wave pattern may have its direction at right angles to or run parallel to the direction of ciliary motion (Knight-Jones, 1954).

The general direction of mucus transport from the upper and lower airways is towards the pharynx. However, specific m.c. streaming patterns such as whirlpool formations, areas of stasis, focal derangements of the metachronical wave pattern, reversal of transport directions and abnormal patterns of mucus production have been described (Hilding, 1957; Iravani, 1967 a; Iravani & Leymann, 1969; Iravani, 1973; Wanner, 1977).

The mechanism responsible for controlling ciliary beating and coordination between adjacent cilia has not yet been established (see Asmundsson & Kilburn, 1973; Wanner, 1977).

B. Mucus secretory apparatus

The presence of a mucus blanket with appropriate volume and viscoelastic properties is essential for the successful functioning of the m.c. activity (Litt, 1970; Yeager, 1971; Boyd, 1972; Dulfano, 1973). According to Lucas & Douglas (1934) and Sturgess (1977) the secretion that covers the epithelium is composed of two layers: an inner "sol" layer of serous fluid in which the cilia beat, and an overlying viscoelastic "gel" layer of mucus. A question of controversy is whether this blanket of mucus is continuous (Lucas & Douglas, 1934; Hilding, 1957) or discontinuous (Van As & Webster, 1974). According to Sturgess (1977) the ciliated epithelium in the rabbit airways is most likely covered with a continuous gel layer.

The origin of the sol layer is not clarified (Reid & Jones, 1979). It could be the product of as yet unidentified epithelial cells (Reid & Jones, 1979), or the result of active water and solute transport across the respiratory epithelium (Olver et al., 1975), or the result of a liquefying effect of cilia beating on mucus (Negus, 1963) or it may be a polymer mixture of gland and surface epithelial secretion (Edwards, 1978). The gel layer is derived from two sources: secretory cells in the epithelium and submucosal glands (Sturgess, 1977).

The submucosal glands are believed to produce the greater part of the respiratory tract secretion (Reid, 1960). It is difficult to collect specimens separately from the submucosal glands and the epithelial secretory cells in the same animal. It is therefore hard to say if the secretions from the epithelium and the submucosal glands are chemically different and if the two sources are controlled by different mechanisms (Widdicombe, 1978).

C. Nerves and neurotransmitters

The mammalian mucous membranes in the nose and in the paranasal sinuses have a rich sensory innervation from the trigeminal nerve. Efferent impulses are transmitted by parasympathetic as well as by sympathetic nerve fibres (Mitchell, 1954; Malm, 1973, 1974 a, 1974 b, 1977; Änggård, 1974 a, 1974 b; Änggård & Densert, 1974; Änggård & Edwall, 1974).

The preganglionic parasympathetic supply to the upper airways emerge from the brain stem and travel in the pars intermedia and facial nerve through the geniculate ganglion to synapse with postganglionic nerve cell bodies in the pterygopalatine ganglion. From this ganglion postganglionic nerve fibres travel to submucosal glands and blood vessels in the nasal mucosa, Eustachian tube, pharynx and paranasal sinuses (Ishii, 1970, 1980; Cauna et al., 1972; Nomura & Matsuura, 1972). In addition a postganglionic parasympathetic nerve supply from the ciliary ganglion to the ethmoturbinal area has been demonstrated in the feline nose (Malcomson, 1959).

Preganglionic sympathetic nerve fibres to the upper airways have their origin in the lateral horns of the gray matter of the spinal cord. Postganglionic sympathetic nerve fibres originate in thoracic and cervical ganglia of the sympathetic chain. These fibres innervate the submucosal glands and the blood vessels mainly via paravascular plexuses (Dahlström & Fuxe, 1965; Cauna et al., 1972; Änggård & Densert, 1974).

There is disagreement as to whether or not there is direct efferent innervation to the ciliated and epithelial secretory cells of the airways (see Fillenz & Woods, 1970; Staub, 1975). However, nerve fibres have been shown to penetrate the basal membrane of the mucous membrane both in the upper and lower

respiratory tract, each nerve ending in close association with ciliated as well as secretory cells (Jeffery & Reid, 1973). According to the electron microscopic evaluation these efferent fibres were both adrenergic and cholinergic. They have been identified in a number of mammalian species including man and rabbit (Rhodin, 1966; Cauna et al., 1969; Hung et al., 1973; Jeffery & Reid, 1973, 1977).

The general concept of chemical neurotransmission in the parasympathetic nervous system is that acetylcholine promotes transmission both at the pre-ganglionic level, where the transmitter acts on nicotinic receptors, and at the postganglionic level, where the receptors are of the muscarinic type (see Koelle, 1975).

In the sympathetic nervous system noradrenaline is released at the postganglionic level whereas acetylcholine promotes transmission at the preganglionic level, where the receptors are of the nicotinic type (see Koelle, 1975).

Recent studies have demonstrated a rich supply of peptide containing nerve cells in the upper respiratory tract. Among such fibres, those containing vasoactive intestinal polypeptide (VIP) and substance P are numerous around the blood vessels and submucosal glands (Uddman et al., 1978, 1980; Lundberg et al., 1981; Uddman et al., 1981). In addition substance P fibres are numerous in tissues known to receive a heavy sensory innervation (Hökfelt et al., 1975), and there is evidence that a population of the substance P fibres may represent the peripheral ramifications of sensory neurons (Gamse et al., 1980; Änggård, 1981).

D. Nervous and pharmacological influences on the mucociliary apparatus

From early investigations it was concluded that the m.c. activity functions independently of nervous and humoral control (Hill, 1928; Lucas & Douglas, 1935). This view has been revised, and in a number of in vitro and in vivo investigations in man and in the experimental animal, both parasympathomimetic and sympathomimetic agonists have been shown to influence m.c. activity. However, the reported effects of such drugs are contradictory. Both accelerated and decelerated ciliary beat frequency, increased and decreased mucus flow rates have been demonstrated (for details and references see II and III).

Cholinoceptor and adrenoceptor antagonists have also been ascribed contradictory effects on the m.c. activity, and it is not certain whether the resting m.c. activity is under a parasympathetic or sympathetic tone (for details and references see II and III).

Nervous and pharmacological influences on m.c. activity can be due to actions on either the mucus secreting apparatus, resulting in changes in the rheological properties of the secretions that cover and surround the cilia, or on the ciliated cells. Effects on isolated ciliated cells are extremely difficult to study in the mammalian respiratory tract (c.f. Corssen & Allen, 1959), whereas the effects on the mucus secreting apparatus are better known.

The resting secretion from the submucosal glands of the feline trachea continues after vagotomy and after administration of cholinoceptor and adrenoceptor blocking substances (Gallagher et al., 1975). The secretion is also present in organ culture preparations (Sturgess & Reid, 1972). It has therefore been

suggested that the basal secretion continues independently of innervation, and that the autonomic nerves increase the rate of secretion from the resting level (Widdicombe, 1978). Stimulation of the vagus nerve promotes secretion of mucus from the submucosal glands of the trachea (Florey et al., 1932; Gallagher et al., 1975; Ueki et al., 1980) and this vagal innervation seems to be cholinergic since the increase in secretion is blocked by the anticholinergic drug atropine and mimicked by injections of cholinergic agonists (Perry & Boyd, 1941; Gallagher et al., 1975). However, this atropine blocking effect has only been noted in the tracheobronchial airways in cats and dogs but not in the rabbit (Boyd, 1954). The reason for this discrepancy between species is unclear. It might be due to the relatively smaller number of submucosal glands present in the rabbit tracheobronchial airways (Haller, 1969) and the large doses of cholinergic agonists needed to promote secretion in the rabbit (Perry & Boyd, 1941; Boyd, 1954).

Sympathetic stimulation promotes secretion of airway mucus in the cat (Gallagher et al., 1975). This secretion is blocked by propranolol and mimicked by injections of isoprenaline, and has therefore been suggested to be mediated via β -adrenoceptors. Noradrenaline promotes secretion (Gallagher et al., 1975), but it is not clear whether also α -adrenoceptors contributed to that response. Thus, both parasympathetic and sympathetic stimulation promote secretion, which suggests a double innervation of the submucosal glands in the cat (Widdicombe, 1978).

The secretory cells of the epithelium are assumed to secrete only as a result of direct irritative stimulation, but not as a result of nervous activation or administration of sympathomimetic or parasympathomimetic agonists (Gallagher et al., 1975;

Wanner, 1977; c.f. Sturgess & Reid, 1973).

The energy supply to the ciliated epithelium is believed to be derived both from ambient air and from the capillary bed (Drettner & Aust, 1977; Reimer et al., 1981), and effects of pharmacological substances on the m.c. activity might be explained in terms of increased or decreased blood flow in the mucous membrane (Staub, 1975). A positive correlation between effect of parasympathomimetic and sympathomimetic drugs on m.c. activity and changes in the blood flow in the mucous membrane has not yet been established (Sackner et al., 1976; Foster et al., 1980). It has been suggested that VIP and substance P plays a physiological role in the neurogenic regulation of blood vessels in the feline nasal mucosa (Änggård, 1979; Malm et al., 1980; Änggård, 1981; Lundberg et al., 1981). Whether these substances can also influence the m.c. activity has not been investigated.

In addition to adrenergic and cholinergic agonists a number of other compounds have been shown to have in vivo or in vitro effects on the m.c. activity e.g. ATP, aminophylline, corticosteroids, morphine derivatives, general and local anesthetics, serotoninine, histamine, antimicrobial drugs, mycolytic agents and cardiac glycosides (see Alexandrov & Arronet, 1956; Gosselin, 1966; Proctor & Adams, 1976; Wanner, 1977).

E. Methods for measuring mucociliary activity

A number of in vivo and in vitro methods have been developed to measure the effect of pharmacological substances on the m.c. activity. Methodological differences may to some extent explain and contribute to the inconsistencies in previously reported data. The methods used may differ both regarding recording techniques and administering procedures.

Recording techniques

M.c. activity can be assessed by basically different methods (Ballenger & Orr, 1963; Mercke, 1974 a; Wanner, 1977; Puchelle et al., 1981). The methods may be divided into different categories depending on whether one records ciliary beat frequency, or more precisely the frequency of mucus waves brought about by the beating cilia (m.c. wave frequency) or mucus flow rate (m.c. transport and m.c. clearance).

Different techniques have been used for recording the m.c. wave frequency, e.g. stroboscopy (Martius, 1884; Gray, 1931; Ballenger & Orr, 1963; Andersen, 1971), photography (Gray, 1931; Frenckner & Richtn r, 1939; Dalhamn, 1956; Iravani 1967 b) and with the aid of a photo-sensitive cell (Dalhamn & Rylander, 1962; H kansson & Toremalm, 1965; Guillerm et al., 1965; Mercke et al., 1974 a; Toremalm et al., 1974; Reimer & Toremalm, 1978).

Stroboscopic methods do not register rapid, irregular frequency changes. Cinematographic methods are time-consuming and expensive for routine experiments. They are also less appropriate for in vivo situations and recordings for long periods of time. The photo-electric recording technique on the other hand offers several advantages in that shallow and rapid alterations in the m.c. activity can be registered and the activity can be measured continuously for long periods of time. Another advantage is that no markers need to

be introduced during the recordings (see Håkansson & Toremalm, 1965; Dalhamn, 1970 a; Mercke et al., 1974 a). This recording technique has been chosen for the investigations reported in this thesis.

M.c. transport can be measured as the time needed for a marker placed on the mucus to be transported a given distance or as the distance a marker is moved during a fixed period of time. The transport technique has been widely used for analysing m.c. activity (Sharpey, 1835; Hill, 1928; Hilding, 1932; Messerklinger, 1951; Dalhamn, 1956; Ewert, 1965; Simon et al., 1977). M.c. clearance is measured as the removal of a known amount of deposited or inhaled markers. This approach, in its modern design, has been developed by Baetjer & Bates (1966), Albert et al. (1967), Morrow et al (1967), Rylander (1968), Quinlan et al. (1969) and Camner et al. (1971 b).

Results obtained by m.c. transport measurements are difficult to compare with those obtained by m.c. clearance techniques, mainly because of inherent methodological variations (Wanner, 1977). Most markers or tracers used to measure m.c. transport (e.g. poppy seeds, bacteria, teflon discs, cell debris, air bubbles) are relatively large and do not penetrate into the mucus layer, and may thus reflect surface mucus velocity. Tracers used to measure m.c. clearance rate are often smaller in size and administered as aerosol inhalations. There is a possibility that the smaller tracers might penetrate into the deeper layer of the mucus or periciliary fluid. It has been suggested that markers might be transported at different velocities depending on whether they are deposited on the surface of the mucus or whether they are dissolved in the mucus (Albert et al., 1967; Chopra et al., 1977; Wanner, 1977). Another difference is that m.c. clearance by aerosol techniques measures the activity in whole organs, whereas m.c. transport measures the m.c. activity in anatomically defined

parts of the ciliated epithelium (c.f. Foster et al., 1980). M.c. clearance rate depends on the deposition pattern, and tracers deposited in the central airways are more rapidly eliminated than tracers deposited in the distal airways (Morrow et al., 1967; Albert et al., 1973). Respiratory rate, tidal volume and coughing also influence the results (Wanner, 1977). The direction and the velocity of mucus flow differs in the same organ (see section A), and such differences contribute to the difficulties in measuring the effect of pharmacological substances on the m.c. activity as changes in m.c. transport rate.

Administering procedures

Test substances can be administered either locally (i.e. in drop or aerosol form or by dipping the ciliated membrane in a test solution) or parenterally (Rylander, 1966). Local administration minimizes general side effects and imitates the local medication often prescribed in clinical practice. However, local application is not believed to imitate the natural physiological route by which m.c. apparatus is normally controlled in the airways. It is also a disadvantage that local application causes tactile stimulation and might change the rheological properties of the mucus (Maxwell, 1905; Hill, 1957; Rylander, 1966; Yeager, 1971). Moreover, it is difficult to administer reproducible doses of the test substances to the effector cells via this route. Most of these disadvantages can be eliminated by parenteral administration of test substances. Different routes for parenteral administration are available, but they can only be utilized in in vivo experiments.

DEFINITION OF PROBLEMS AND AIMS OF THE STUDY

Several drugs commonly prescribed in medical practice have their action on cholinceptors and adrenoceptors in the autonomic nervous system. The influences on the m.c. activity of parasympathomimetic and sympathomimetic agonists and antagonists have been studied for decades but several questions still remain unanswered and reported data are often contradictory. Some possible reasons for this are that measuring methods may be burdened with limitations and that test models have not been specifically adapted to the study of pharmacological effects on the m.c. activity. To achieve as physiologically correct test conditions as possible special demands must be imposed upon the test model used. Thus, fundamental principles regarding environmental conditions must be respected and disturbances to the observed part of the mucous membrane (caused by the recording technique as well as by the administering procedure of test substances) must be reduced to minimal proportions. Only when a method fulfilling these basic demands has been designed and standardized are investigations of the effect on the m.c. activity of various pharmacological substances possible. From both the physiological and the clinical point of view substances having their action on receptors in the autonomic nervous system would be of primary interest.

The aims of the present study have therefore been:

1. To develop and to standardize an in vivo method for evaluating the influences of pharmacological substances on m.c. activity (I).
2. To study the effect on the m.c. activity of:
 - parasympathomimetic agonists and antagonists (II),
 - sympathomimetic agonists and antagonists (III),
 - the nicotine in cigarette smoke (IV).

THE PRESENT INVESTIGATIONS

A method for evaluating the effect of pharmacological substances on mucociliary activity in vivo (I).

The inconsistencies in earlier reported data concerning the effect of pharmacological substances on the m.c. activity may be related to the use of recording techniques burdened with experimental limitations, to insufficient attention taken to principles concerning environmental conditions and to the use of inappropriate administering procedures of test substances.

The purpose of the present investigation was therefore to develop and standardize a new test model designed to meet the following demands:

1. To register transient and shallow fluctuations in m.c. activity
2. To make recordings for a long period of time
3. To eliminate external sources of error during recording
4. To minimize disturbances to the mucous membrane and its mucus sheet
5. To administer test substances to the mucous membrane via the blood vessels
6. To record under in vivo condition

Procedure. The m.c. activity was recorded in a limited area of the rabbit maxillary sinus mucosa by a photo-electric technique. The test model is described in detail in I, and the experimental model is shown in Fig. 1 (page 19).

In order to standardize the experimental procedure and to study the functional state of the test model and the reproducibility of the responses the following factors were analyzed:

1. Anesthesia
2. Sealing of the trepanation hole with an antimist window
3. Temperature of the antimist window
4. Respiratory and cardiac interference
5. Arterial catheterization and perfusion of the cannula
6. Volumes and injection velocities of the test substances
7. The basal m.c. wave frequency in the maxillary sinus in anesthetized rabbits
8. The reproducibility of the responses to some drugs previously reported to influence the m.c. activity

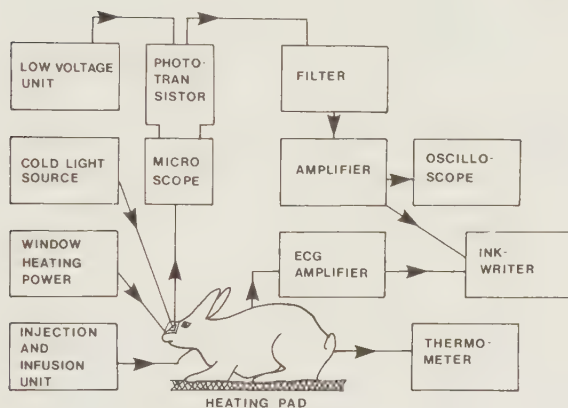


Fig. 1. Block diagram of the experimental set up. For details see Methods (I).

Results. The anesthetic drug used (urethane) gave a satisfactory depth of anesthesia for several hours and had no influence on the m.c. activity. Sealing of the trepanation hole preserved the fluctuations of the resting m.c. activity within narrow limits. To avoid condensation on the inside of the window it was heated to just above the body temperature of the rabbit. The window temperature fluctuating within 3°C above body temperature did not disturb the resting m.c. activity. Disturbances of respiratory or cardiac origin could be eliminated. Catheterization of the artery supplying

the sinus mucosa and perfusion of this catheter did not influence the m.c. activity. The average basal m.c. wave frequency (66 rabbits) was 1228 ± 174 (mean $\pm$ S.D.) waves/min. Tests with some drugs (e.g. parasympathomimetics and sympathomimetics) showed that the responses were dependent on the injection procedure, i.e. the volumes and the injection velocities of the test substances, therefore these two factors have been standardized.

Conclusions. An in vivo test model has been developed which satisfies the demands initially set up. It permits easy detection of shallow fluctuations in the m.c. activity of both short and long duration. There is minimal exposure to external disturbance since the m.c. wave frequency is measured with a non-invasive (photo-electric) technique, the normal sinus ventilation is retained and the test solutions reach the investigated mucous membrane in a way that does not disturb its covering mucus sheet.

Effects of the parasympathomimetic drug methacholine and its antagonist atropine on mucociliary activity (II).

The parasympathetic nervous system is believed to play an important role in the airway defence system. The action of parasympathomimetic agonists on the m.c. activity is not, however, well-known. It has been proposed that such substances do not play a physiological role in the control of ciliary activity in mammals; the reported effects have been small and the doses required to affect the activity quite large. It is also uncertain as to whether and to what extent the resting m.c. activity is dependent on parasympathetic basal or reflex activity, since anticholinergic drugs have been reported both to stimulate and to depress the resting m.c. activity.

The purpose of the present investigation was to study the effects of parasympathomimetic agonists and antagonists on the m.c. activity with the aid of the test model described in paper I.

Procedure. The material consisted of 39 rabbits. The experimental design is described in I and II. The parasympathomimetic drug methacholine and its antagonist atropine were given close intra-arterially in different doses as 0.2 ml, 3 sec bolus injections. Saline injections at the same volume rates served as controls.

Results. Methacholine 0.01-2 $\mu\text{g/kg}$ i.a. gave a dose-dependent increase in the m.c. activity (Fig. 2, page 25). The threshold dose was calculated to be 0.03 $\mu\text{g/kg}$. Atropine 0.05-0.5 mg/kg i.a. did not influence the basal m.c. wave frequency, but in a dose of 0.2 mg/kg it reduced or abolished the response induced by methacholine (0.05-2 $\mu\text{g/kg}$).

Conclusions. The responses to methacholine indicate the presence of muscarinic receptors on effector cells that may either directly or indirectly be involved in the control of the m.c. activity. Together with the earlier described findings of parasympathetic nerve fibres in close proximity to the respiratory epithelium, the present results suggest a parasympathetic nervous control of m.c. activity. Apparently the resting m.c. activity functions independently of parasympathetic nervous activity in the anesthetized rabbit since atropine did not influence the basal m.c. wave frequency.

Effects of sympathomimetic agonists and antagonists on mucociliary activity (III).

Information about the effect of sympathomimetic substances on m.c. activity is sparse and often conflicting. In several earlier studies fairly non-selective agonists, acting on more than one of the now well-known different adrenoceptor types have been used. The modern receptor concept indicates that there are different groups of receptors (α_1 , α_2 , β_1 and β_2 -adrenoceptors) with which sympathomimetic agonists can react to elicit a response in the effector cells. There is only limited information in the literature regarding the effects of selective agonists on m.c. activity, nor is it known if the resting m.c. activity is dependent on a basal or reflexly evoked activity in the sympathetic nerves.

The purpose of this investigation was to study (1) the effect on m.c. activity of adrenoceptor stimulating substances with selectivity to different kinds of adrenoceptors, (2) whether sympathomimetic antagonists influence m.c. activity, and (3) whether such antagonists influence the effect of α and β -adrenoceptor agonists, respectively.

Procedure. The effect of different doses of sympathomimetic agonists and antagonists on m.c. activity was studied in 43 rabbits, prepared and investigated as described in I and II. To test the functional state of the test model the parasympathomimetic drug methacholine was given. Controls with physiological saline solution were also employed.

Results. The selective β_1 -adrenoceptor agonist prenalterol did not influence the resting m.c. activity. The β -adrenoceptor agonists salbutamol (β_2) and isoprenaline (β_1 and β_2) were both found to give a dose-dependent increase of the wave frequency (Fig. 2, page 25). The non-selective β -adrenoceptor

antagonist propranolol did not per se influence the resting m.c. activity, but it reduced the effects of both salbutamol and isoprenaline.

The α -adrenoceptor agonists phenylephrine (α_1) and oxymetazoline (α_2) both gave a dose-dependent decrease in the m.c. activity (Fig. 2, page 25). The retarding effect of oxymetazoline was abolished by the α -adrenoceptor antagonist phentolamine, which on its own did not influence the resting m.c. activity. When injections of oxymetazoline or phenylephrine were repeated within 10-20 min the second dose was found to be without effect. Similarly methacholine given within the same time limits after administration of oxymetazoline was without effect.

Conclusions. Sympathomimetic agonists with selectivity to different groups of adrenoceptors have opposite effects on the m.c. activity, i.e. acceleration by β_2 and retardation by α_1 and α_2 -adrenoceptor agonists. The resting m.c. activity was found to function independently of sympathetic activity in the anesthetized rabbit since β and α -adrenoceptor antagonists on their own did not influence the resting m.c. activity.

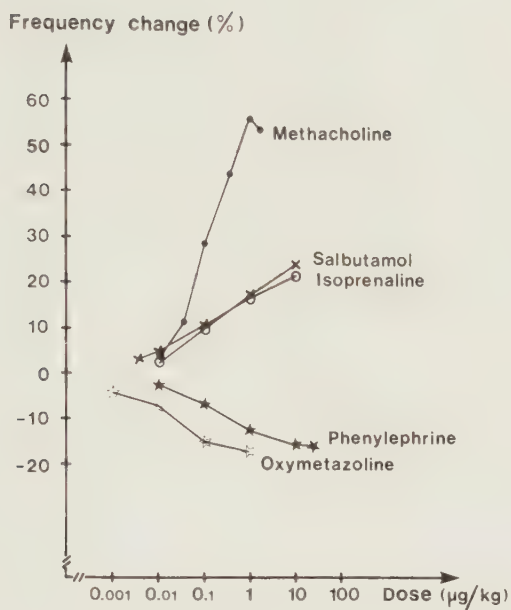


Fig. 2. The effect on the m.c. wave frequency of i.a. injections of different doses of the parasympathomimetic drug methacholine and of the sympathomimetic drugs salbutamol (β_2), isoprenaline (β_1 and β_2), phenylephrine (α_1) and oxymetazoline (α_2). For details of differences in injection velocities see II and III.

A pharmacological evaluation of the short-term effect of cigarette smoke on mucociliary activity (IV).

Cigarette smoke is believed to play an important role in the etiology of airway diseases. Earlier investigations have shown conflicting effects on m.c. activity after short-term exposure to cigarette smoke. Both increase and decrease of the m.c. activity as well as no effect at all have been reported.

The nicotine in the cigarette smoke is absorbed from the mucous membranes in the upper airways almost with the efficacy of i.v. administration. A pharmacological effect of cigarette smoke on m.c. activity can thus be assumed to be mediated by its nicotine content. The nicotine dose available to a smoker from a cigarette depends not only on the cigarette specification but also on the way in which the cigarette is smoked. There seems to be no previous study undertaken to show the effect of nicotine in the cigarette smoke during blockade of nicotinic receptors.

The intention has therefore been to study (1) the effect of cigarette smoke delivered from cigarettes of various nicotine content and smoked in a way that imitates the normal smoking habit in man, and (2) the effect of cigarette smoke after blockade of receptors in the autonomic nervous system.

Procedure. Anesthesia, surgical procedure and recording technique were as in I, II and III. Cigarette smoke was delivered to 22 rabbits in 3.5 ml puffs once every min. The smoke was drawn from 3 different cigarette types, one nicotine free and the other two containing 0.6 and 1.8 mg nicotine per cigarette, respectively. Ganglion nicotinic receptors were blocked with hexamethonium and postganglionic muscarinic receptors with atropine.

Results. Cigarette smoke accelerated the m.c. wave frequency and this effect was positively correlated with the nicotine content of the cigarettes used. The accelerating effect of cigarette smoke was reduced by hexamethonium (Fig. 3, page 28) and by atropine (Fig. 4, page 28).

Conclusions. The accelerating effect on the m.c. activity can be explained by the nicotine in the smoke, as the effect was dose-dependent and as the effect could be blocked by hexamethonium. Nicotinic receptors are situated both on parasympathetic and on sympathetic nerve cell bodies. Stimulation of parasympathetic nerve cells seems to be responsible for the accelerating effect, as atropine blocked the action of cigarette smoke on the m.c. activity. Stimulation of sympathetic nerve cells on the other hand is probably not responsible for the smoke effect as a release of noradrenaline does not accelerate the m.c. wave frequency (see I and III). Reflexly activated cilia in the sinus cavity due to local irritation does not seem to be responsible for the responses because nicotine free cigarettes did not influence the resting m.c. wave frequency.

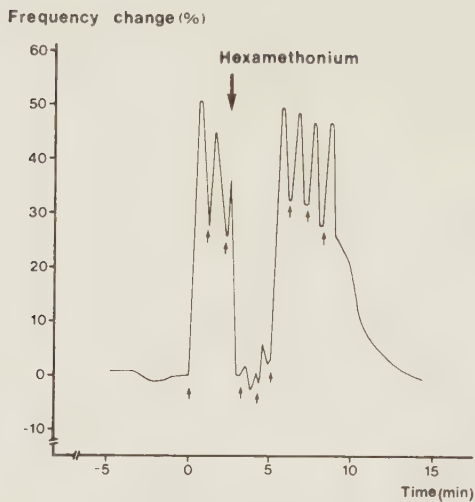


Fig. 3. The effect on the m.c. wave frequency in one rabbit when exposed to a series of smoke puffs drawn from a cigarette containing 1.8 mg nicotine. Arrows under the curve indicate each puff. Hexamethonium 0.2 mg/kg i.a. was given at the big arrow over the curve. The ordinate represents frequency change expressed as a percentage of the resting wave frequency at the instant the smoke exposure commenced. Frequency zero level corresponds to 1236 waves/min.

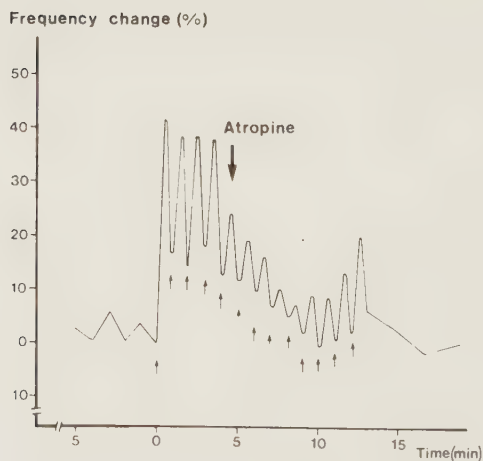


Fig. 4. The effect on the m.c. wave frequency in one rabbit exposed to a series of smoke puffs. Atropine 0.2 mg/kg i.a. was given at the big arrow over the curve, ordinate and cigarette specification are as in Fig. 3. Frequency zero level corresponds to 1356 waves/min.

GENERAL DISCUSSION

In the m.c. system the cilia beat incessantly, thereby propelling the mucus and the embedded particulate impurities towards the pharynx. The system is thus of decisive importance for the defence of the organism against toxic substances and microorganisms contained in inhaled air.

The aims of the present investigation were to develop a test model for studying the effect of pharmacological substances on m.c. activity and then to investigate the influences on the m.c. activity of drugs acting on the parasympathetic and the sympathetic nervous system.

Methodological considerations

The method developed (I) is an in vivo model for studying the effects of pharmacological substances on the m.c. activity in the rabbit maxillary sinus.

Different methods for recording m.c. activity have been reported in the literature (see section E). The application of the photo-electric recording principle for this purpose was first presented by Dalhamn & Rylander (1962) and improvements and modifications have been made by Håkansson & Toremalm (1965) using a TV-camera, by Mercke et al. (1974 a) using a photo-multiplier and by Reimer et al. (1977) using a photo-transistor. The photo-electric technique is non-invasive, needs no tracer substances and permits momentary recording of rapid and shallow fluctuations of the m.c. activity during long periods of time. With the photo-electric technique recordings can be made from a constant and limited area of the mucous membrane (Reimer et al., 1977). Because of these advantages this technique was chosen to constitute the recording part of the present animal test model.

Environmental disturbances such as variations in temperature and humidity in the ambient air have been shown to influence m.c. activity (Mercke, 1974 b; Mercke et al., 1974 b; Mercke, 1975; Mercke & Toremalm, 1976).

To avoid heating of the investigated mucous membrane by the illumination during the photo-electric recording a cold light source was utilized (I) instead of the original lamp in the operating microscope. To avoid noxious effects from unconditioned air, the trepanation hole to the sinus cavity was sealed with an antimist window and bone wax. This restored the sinus ventilation to normal so that constant and optimal humidity and temperature conditions could be maintained for long periods in the sinus cavity, thereby keeping the fluctuations in the resting m.c. activity within narrow limits. Experiments showed a complete cessation of the sinus m.c. activity after breathing room air through an open trepanation hole for about 20 min (I). Even a small leakage in the sealing gave a decrease in the sinus m.c. activity.

Tactile stimulation and changes in rheological properties of the respiratory secretion also influence the m.c. activity (see section B and E). Local application of test solutions was therefore avoided, and parenteral administration was selected for the present test model, the i.a. route being used most. Compared to i.v. administration it has the advantage that smaller doses can be given for equal responses, which has been shown in the cat and rat salivary gland (Emmelin et al., 1970; Thulin, 1974, 1976). General side effects can also be minimized.

The ciliated epithelium in the maxillary sinus was selected for observation, since the mucous membrane in this location being supplied by one major artery, the maxillary artery (Jeppsson & Olin, 1960). In addition, the membrane in the sinus cavity rests

against a bone wall, which is of importance for mechanical stability during in vivo photo-electrical recording of m.c. activity.

In order not to disturb the blood supply and the innervation of the sinus membrane the i.a. cannula used for administering the test substances was advanced via the facial or lingual artery and not the maxillary artery. The sympathetic nervous plexus surrounding the carotid and maxillary artery (Dahlström & Fuxe, 1965; Malm, 1974 b) was thus left intact and the blood flow through the maxillary artery was virtually unaffected.

Tests with the present experimental model (I) and experiences obtained during the succeeding investigations (II, III and IV) have shown that the model fulfills the requirements originally set out (I). Thus, rapid and shallow changes in the m.c. activity could be detected (see effects of methacholine in I and II and of cigarette smoke in IV). The wave frequency could be recorded continuously for several hours, and repeated injections of the test substances could be administered to the same animal (see I, II, III and IV). Environmental disturbances to the investigated mucous membrane were minimized both during recordings of the activity and during the administering procedures and it was also possible to perform the experiments under in vivo conditions. In addition, the model ensures that recorded changes in the m.c. activity are dependent on the drug administered and not on existing variations in the activity between adjacent areas of the ciliated epithelium (see section A).

There are variations between different species and organs in the arrangement of different epithelial cell types, in the distribution and composition of sub-mucosal glands, in the density of ciliated cells and in the length of their cilia (Haller; 1969, Jeffery, 1978; Reid & Jones, 1979). The significance of these

variations in connection with the resting m.c. activity or the influence of pharmacological substances on the m.c. activity is not known (Lucas & Douglas, 1934; Perry & Boyd, 1941; Boyd, 1972; Andersen et al., 1974; Wanner, 1977). These variations may, however, contribute to some of the discrepancies in earlier reported data in different animal test models.

In the present investigation, effects of pharmacological substances on m.c. activity were measured as changes in m.c. wave frequency, whereas hitherto most comparative studies in the literature have measured changes in mucus flow rate. A positive correlation between ciliary beat frequency (or more correctly m.c. wave frequency) and mucus flow rate has been shown by Blair & Woods (1969) and H  e & Guillerm (1973). However, this correlation was not significant (Andersen, 1971).

The relation between mucus flow rate, ciliary beat frequency and the respiratory tract secretion can be interpreted in terms of the Lucas-Douglas model (Lucas & Douglas, 1934) according to which decrease of mucus flow rate could be the result of either decelerated ciliary beat frequency or of clogging of the cilia by the gel due to reduced sol layer thickness. In addition, mucus flow rate can be decreased even with normal ciliary beat frequency. This could be due to excessive thickness of the sol layer, since this will raise the gel which then loses its contact with the beating cilia.

Pharmacological substances might influence both the ciliated cells and the mucus secreting apparatus. The Lucas-Douglas model serves to illustrate the difficulties in determining the sites of action of test substances when only changes in mucus flow rate or in m.c. wave frequency (which is measured with the present test model) are known.

Pharmacological considerations

Resting m.c. activity continues for a long period in denervated in vitro preparations (Håkansson & Toremalm, 1965) and in in vivo experiments after vagotomy (Lucas & Douglas, 1935; Dalhamn, 1970 b). Spontaneous activity is also present after blocking of nicotinic receptors in autonomic ganglia (IV), of postganglionic parasympathetic muscarinic receptors (II) and of postganglionic sympathetic α and β -adrenoceptors (III). It might therefore be suggested that the resting m.c. activity functions independently of parasympathetic and sympathetic innervation.

Drugs acting on postganglionic muscarinic receptors (e.g. methacholine in II), on postganglionic β_2 -adrenoceptors (e.g. salbutamol and isoprenaline in III) and on ganglion nicotinic receptors (e.g. nicotine in cigarette smoke in IV) accelerate the m.c. wave frequency in a dose-dependent manner. This accelerating effect is antagonized by receptor blocking substances, e.g. atropine, propranolol and hexamethonium (II, III and IV). It might thus be concluded that the role of the autonomic nervous innervation of the mucous membrane, in addition to the established effects on airway smooth muscles, mucus secreting apparatus and blood vessels (Widdicombe et al., 1962; Ånggård, 1974 b; Malm, 1974 b; Gallagher et al., 1975) is to increase the m.c. activity.

In the present investigation there is no evidence that inhibitory receptors exist on the m.c. apparatus. However, agonists acting on α_1 and α_2 -adrenoceptors (phenylephrine and oxymetazoline in III) were found to decelerate the resting m.c. wave frequency. Nevertheless, the α -adrenoceptor antagonist phentolamine did not accelerate the resting m.c. wave frequency (III) despite the fact that the blood vessels in the mucous membrane are believed to be under a continuous

α -sympathetic tone, shown for example in the anesthetized cat (Änggård & Densert, 1974; Malm, 1974 a). α -adrenoceptor agonists decrease the blood flow in the nose (Malm, 1974 a) and it is possible that the retardation of the m.c. wave frequency following injections of phenylephrine and oxymetazoline (III) reflects a decrease in energy supply to the ciliated epithelium (c.f. Reimer et al., 1981). However, α -adrenoceptors situated on the ciliated cells or on the mucus secreting apparatus cannot entirely be excluded (see section D).

From the present data, the mechanisms responsible for the accelerating effect of parasympathomimetic agonists (II), β_2 -adrenoceptor agonists (III) and the nicotine in cigarette smoke (IV) cannot be evaluated. Nerve stimulations were not performed. The present results therefore provide only circumstantial evidence of a true motor innervation of the ciliated epithelium.

The responses following injections of methacholine (II) and exposure to cigarette smoke (IV) were characterized by short latency, short duration and reproducibility within short time limits. In contrast, earlier reported data concerning parasympathomimetically induced changes in the rheological properties of mucus do not have such characteristics (c.f. Reasor et al., 1978; King & Viires, 1979). This might imply that the observed effect of parasympathomimetic drugs on the m.c. activity was not mediated via the mucus secreting apparatus but by action on the ciliated cells.

Compared to methacholine (II), isoprenaline and salbutamol (III) were less effective in accelerating the m.c. activity, and the responses came after a long latency. Whether these differences can be attributed to different effector cells for the two kinds of agonists cannot be evaluated from the

present data since only changes in m.c. wave frequency were measured (c.f. Staub, 1975; Foster et al., 1980).

In contrast to the present results, Mossberg (1977) in a review of earlier investigations suggested that m.c. activity was increased more by β_2 -adrenoceptor agonists than by parasympathomimetic agonists. However, this proposition is based on reported effects of single doses of agonists, whereas the present results are based on a wide range of doses and the maximum effects of such agonists as derived from dose-responses correlations (Fig. 2, page 25).

Clinical considerations

Even though results from animal experiments cannot be directly transferred to clinical practice, the present investigation is supported by observations in man. Parasympathomimetics, sympathomimetics and cigarette smoke have all previously been shown to increase pulmonary m.c. transport and clearance rate in man (e.g. Camner et al., 1971 a; Camner et al., 1974; Santa Cruz et al., 1974; Albert et al., 1975). Also, the retarding effect of α -adrenoceptor agonists is supported by clinical observations in the human nasal airways (Simon et al., 1977). The action of these substances on m.c. activity in the paranasal sinuses does not seem to have been investigated before, neither in man nor in animal test models.

In the present investigation only healthy rabbits were examined, whereas the effect in disease is of special interest in clinical practice. Abnormal autonomic function has been demonstrated in patients suffering from allergy and cystic fibrosis, where diminished β -adrenergic and exaggerated cholinergic responses have been observed (Kalinin, 1980). In addition, some reports indicate differences in the magnitude of the responses to β -adrenoceptor agonists on pulmonary m.c. activity between healthy and diseased subjects (c.f.

Santa Cruz et al., 1974; Camner et al., 1976; Mossberg et al., 1976).

Not only the m.c. activity, but also other protective mechanisms in the human lung (e.g. coughing and bronchoconstriction) are believed to be facilitated or mediated by reflexly evoked activity in parasympathetic vagal nerve fibres (Widdicombe et al., 1962; Albert et al., 1973; Camner et al., 1974; Nadel, 1980). Hence, it cannot be excluded that anticholinergic drugs have adverse effects not only on parasympathomimetically induced improvements in m.c. activity (II) but also on other airway defence mechanisms (c.f. Jackson & Teichgraeber, 1981).

β_2 -adrenoceptor agonists have an established role in the therapy of hyperreactive pulmonary airways and have been shown to increase the m.c. activity in the pulmonary airways (Santa Cruz et al., 1974; Camner et al., 1976). In addition to these effects, β_2 -adrenoceptor agonists might be valuable adjuncts in the m.c. defence system in the upper airways (III).

α -adrenoceptor agonists are widely used as vasoconstrictors in the treatment of nasal congestion (Aust et al., 1979; Gilman et al., 1980; Axelsson et al., 1981). Information about their influence on the m.c. activity is sparse and often conflicting (Proctor & Adams, 1976). In clinical studies such decongestants have been claimed to play a causative or precipitating role in the etiology of otitis media with effusion (Peerless & Noiman, 1980). The present study (III) reveals evidence that α -adrenoceptor agonists interfere with normal m.c. activity.

The effect of cigarette smoke on m.c. activity is of clinical importance, and several constituents in tobacco smoke are believed to cause airway diseases (Wanner, 1977). The deleterious effect of cigarette smoking in man is often only obvious after decades,

whereas in animal experiments short-term exposure has also been claimed to result in ciliostasis and arrest of mucus flow rate (Dalhamn, 1970 b; Isawa et al., 1980). The experimental effect on m.c. activity depends on the administering procedure (Dalhamn & Rylander, 1969). It is also possible that the effect of cigarette smoke might vary in different regions of the respiratory tract depending on whether or not the mucous membrane is directly exposed to unconditioned smoke. In the present study tobacco smoke was found to accelerate the m.c. activity in the maxillary sinus (IV). The gas exchange between the nose, which was exposed in the present study, and the sinus cavity is assumed to be small (Drettner & Aust, 1977). Hence, the present data cannot be transferred directly to other parts of the respiratory tract. Further, the present data are only based on short-term exposure and the effect in the long run might be quite different.

GENERAL SUMMARY

A new animal test model for studying the effect of pharmacological substances on m.c. activity is presented.

The model permits administration of test substances and recording of m.c. activity under conditions that closely mimic the normal physiological situation. Various factors which may influence the results, e.g. the anesthesia, disturbances of cardiac or respiratory origin and drug administering procedure are analyzed and discussed. The experimental model permits easy detection of changes in m.c. activity and gives reproducible results.

The parasympathomimetic drug methacholine induced a dose-dependent acceleration of the m.c. wave frequency, whereas its antagonist atropine did not on its own influence the resting m.c. activity, but reduced the magnitude of the effect of methacholine.

Sympathomimetic β_2 -adrenoceptor agonists (salbutamol and isoprenaline) accelerated the m.c. activity dose-dependently, whereas β_1 -adrenoceptor stimulation (prenalterol) was without effect. Agonists acting on α_1 and α_2 -adrenoceptors (phenylephrine and oxy-metazoline) retarded the m.c. wave frequency dose-dependently. β and α -adrenoceptor antagonists (propranolol and phentolamine) did not on their own influence the resting m.c. activity, but reduced the effect of β and α -adrenoceptor agonists, respectively. α -adrenoceptor agonists were also shown to reduce the magnitude of the accelerating effect of methacholine, but they had no influence on their own when given in cumulative doses.

A pharmacological evaluation of the short-term effect on m.c. activity of cigarette smoke delivered in a way that imitates normal smoking in man showed that

the m.c. frequency was accelerated, and that the magnitude of this acceleration was positively correlated with the nicotine content of the smoke. The effect of cigarette smoke was blocked by antagonists acting on ganglion nicotinic receptors (hexamethonium) and on postganglionic parasympathetic muscarinic receptors (atropine).

In conclusion, the resting m.c. activity during anesthesia functions independently of parasympathetic and sympathetic innervation. The m.c. activity is accelerated by parasympathomimetics, β_2 -sympathomimetics and by stimulation of nicotinic receptors located on postganglionic parasympathetic nerve cell bodies. α -sympathomimetics retard the m.c. wave frequency. The possible sites of action and the possible clinical utility of these substances are discussed.

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A METHOD FOR EVALUATING THE EFFECT OF PHARMACOLOGICAL SUBSTANCES ON MUCOCILIARY ACTIVITY IN VIVO

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Abstract. A test model for in vivo studies of the effect of pharmacological substances on mucociliary activity in the rabbit maxillary sinus is presented. The method permits administration of pharmacological substances and recording of the mucociliary activity under conditions that mimic the physiological situation very closely. The test substances are given intra-arterially. Using a photoelectric technique the mucociliary activity is recorded from a small and limited area of the mucous membrane. There is minimal exposure to external stimuli since normal sinus ventilation is retained. Various factors which may influence the results, e.g. the anesthesia, movements from cardiac and respiratory activity, and the volumes and injection velocities of test doses, are analysed and discussed. When the animal model was tested with serotonin, ATP, and certain cholinergic and adrenergic agents, it was found to be reliable. It permitted easy detection of changes in the mucociliary activity and gave reproducible results.

The mucociliary (m.c.) transport system is an important defence mechanism in mammalian airways. Impaired or lost transport capacity can contribute to chronic or recurrent infection, which is illustrated in patients with chronic bronchitis and non-functioning cilia (Camner et al., 1973; Afzelius, 1979).

From early investigations it was concluded that m.c. activity functions independently of nervous and humoral control (Hill, 1928; Lucas & Douglas, 1935), a view which has now to some extent been revised. Many pharmacological substances have been found to influence m.c. activity, but the results from different investigations are difficult to compare and are often conflicting (Wanner, 1977).

The inconsistencies in previously reported data may be related to differences for instance in experimental techniques, animal species,

mode and duration of drug administration, and insufficient attention to fundamental physiological principles concerning temperature and humidity, factors investigated by Rylander (1966), Brodie & Reid (1967), Florey (1967), Boyd (1972), Mercke (1974, 1975) and Mercke et al. (1974a, b), among others.

When analysing m.c. activity, it is important to keep the mucous membrane and its mucus layer intact because manipulation of these structures influences the activity (Maxwell, 1905; Hill, 1957; Rylander, 1966). The correct physiological administration of test solutions has therefore constituted a considerable technical and methodological problem. In the in vitro studies reported so far the test substances have been applied locally, i.e. in drop or aerosol form, or by dipping the ciliated membrane in a test solution. In this way the test substances reach the cells in the mucous membrane through the mucus layer, which is not considered to be the natural physiological route. Furthermore, the test solutions will alter the physical properties of the mucus blanket, and hence mechanically influence the recorded m.c. activity. In hitherto described in vivo techniques for registering m.c. activity the test substances are usually administered parenterally, but to detect the effect it is also often necessary to introduce a tracer substance. However, this substance can influence the m.c. activity *per se* and if it is introduced endotracheally as an aerosol it can provoke increased respiratory resistance (Waltemath & Bergman, 1973).

In designing a method for detecting the effects of pharmacological substances on m.c. activity it is important to meet the following requirements:

1. Possibility to register a shallow and rapid alteration in activity.
2. Possibility to make recordings for a long period.
3. Elimination of sources of error during recording where possible.
4. Minimal disturbance of the mucus blanket and mucous membrane.
5. Possibility for the test substance to reach the mucous membrane via the blood vessels.
6. Possibility to record under in vivo conditions.

A method that fulfils all these requirements will permit in vivo pharmacological studies on m.c. activity under as physiologically natural test conditions as possible with environmental disturbances to the recorded mucous membrane minimized.

The aim of the present investigation has therefore been to develop and standardize a test model to meet these requirements. The photoelectric technique for registering m.c. activity previously developed and tested by Mercke et al. (1974*a*) and Toremalm et al. (1975) satisfies the first three requirements listed above. To fulfil the remainder a test model needs to be developed and tested to permit test substances to reach a normal, ciliated membrane via the blood vessels under in vivo conditions.

MATERIAL AND METHODS

Male rabbits of a white strain, raised under controlled conditions and weighing about 2 kg, were used. The animals were anesthetized with urethane (0.25 g/ml) i.m., 2 g/kg as an initial dose and an extra dose of 4–5 ml urethane in an ear vein during the ensuing operation. No further anesthesia was needed for 5–6 hours, and during this period the depth of anesthesia was constant and deep. The criteria for adequacy of anesthesia were regular

spontaneous breathing and no movements by the animal.

The left facial or lingual artery was cannulated (Portex Intravenous Cannula, outer diameter 0.75 mm), the catheter being advanced in the vessel until the tip reached only a few mm from the orifice of the maxillary artery which supplies the mucous membrane in the maxillary cavity. The test substances were given through this catheter and in order to keep it open and to compensate the animal for dehydration it was continuously perfused with physiologic saline solution at a rate of 5–10 ml/hour. The test substances were administered either as an injected bolus dose or as a prolonged infusion.

The maxillary sinus was opened on the same side as the catheterization by drilling a 2×8 mm hole through the bone wall and an incision was made in the mucous membrane. The incised edges of the membrane then retracted to the margins of the trepanation. The hole was immediately covered with a heated glass window through which the ciliated membrane could be studied. The slit between the bone and the glass was sealed with bone wax (Ethicon). Sinus ventilation was thus restored to normal, i.e. only via the nasal cavity. To avoid condensation the window was heated to 1°C above body temperature by means of electrical resistors glued to the glass.

The rabbit's head was stabilized in a specially constructed holder whose position could be adjusted by means of micrometer screws. Light from a cold source (Heim L 151) was directed into the frontosuperior part of the maxillary sinus so that it was reflected from the mucus blanket. The intensity variations in the reflected light, caused by m.c. activity, were observed through an operating microscope (Zeiss, OPMI 1). One eyepiece was replaced with a phototransistor which transformed the light intensity variations into electrical signals. These were bandpass filtered 3–40 Hz (Krohn-Hite 3550) and finally recorded on an ink writer (Elema-Schönander 803). An oscilloscope was used for adjusting

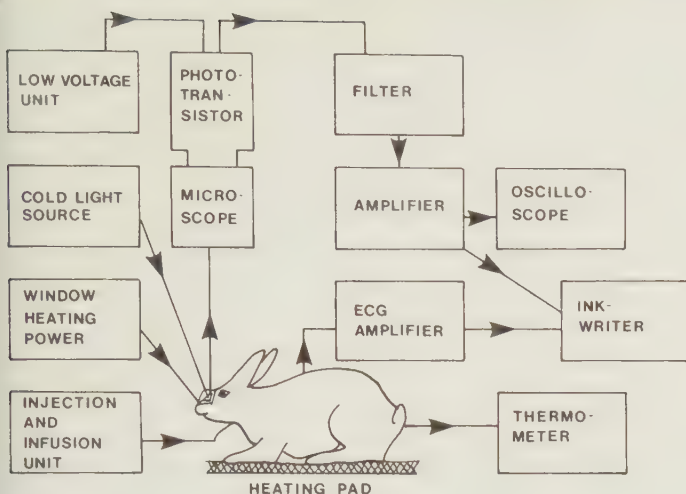


Fig. 1. Block diagram of the experimental set-up. For details, see Methods.

the recorded area and for continuous monitoring.

All experiments were started by recording m.c. activity every 5 min until the wave frequency had remained stable for 20–30 min. The test substance was then administered. Changes in m.c. activity were related to the mean wave frequency during the 20 sec preceding stimulation. The wave frequency was recorded continuously on the ink writer during stimulation and the ensuing 3 min, thereafter at 4, 5, 7 and 10 min after stimulation. If desired recordings could be continued for a longer period. The wave frequencies were averaged over 20-sec intervals.

ECG was also recorded simultaneously with the m.c. activity and in some runs respiratory frequency and arterial blood pressure (right femoral artery) were also noted. Rectal temperature was monitored (Ellab DU-3S and A-RK2) and body temperature was maintained within 37.0–38.5°C with the aid of a heating pad.

To check the correct position of the catheter in the lingual or facial artery the experiments were concluded by injecting a small amount of methylene blue, which should stain the mucous membrane.

The experimental set-up is shown in Fig. 1.

The following drugs were used: urethane (ethyl carbamate), pentobarbital sodium, se-

rotonin (5-hydroxytryptamine creatinine sulphate), ATP (adenosine triphosphate), methacholine chloride, acetylcholine chloride, pilocarpine hydrochloride, adrenalin bitartrate, orciprenaline sulphate (Boehringer Ingelheim), noradrenalin bitartrate.

RESULTS

Detecting a subtle and fleeting effect of a pharmacological agent on m.c. activity requires a strictly standardized method. The following steps have therefore been studied:

1. Anesthesia.
2. Sealing the maxillary trepanation hole with an antimist window, and the temperature of the window.
3. Respiratory and cardiac interference.
4. Arterial catheterization and perfusion of the cannula with physiologic saline solution.
5. Volumes and injection velocities of the test substances.

The anesthetic drug used should have very few general side effects, especially on m.c. activity, and give a constant depth of anesthesia for a long period of time. Barbiturates are often used in *in vivo* experiments, and pentobarbital has been compared with urethane, which proved to be superior in these respects. The effect on m.c. activity of these drugs is exemplified in Fig. 2.

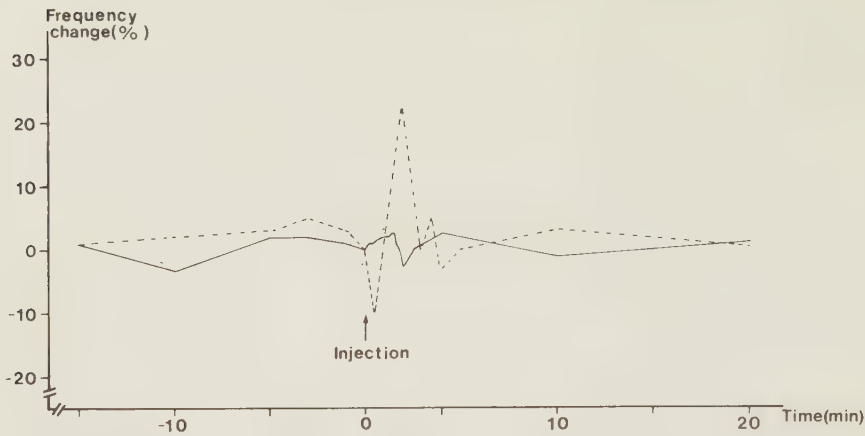


Fig. 2. Effect on m.c. activity in 3 urethanized rabbits when given additional doses i.v. of urethane, 2 ml, 0.25 g/ml (—) and pentobarbital, 0.3 ml, 60 mg/ml (---). Mean

frequency zero level at instant of stimulation was 1245 waves/min and 1137 waves/min, respectively.

Sealing the maxillary trepanation hole with glass and bone wax is essential for preserving normal temperature and humidity in the maxillary sinus. If this cover is neglected the animal will breathe through the maxillary sinus with consequent cooling and drying out of the mucous membrane. Our experiments have shown complete cessation of m.c. activity after breathing through an open maxillary sinus for about 20 min.

The window needs to be heated to just above the normal body temperature of the rabbit (37.0–38.5°C) in order to avoid condensation on the inside surface. Our experi-

ments with the window temperature fluctuating 37–42°C did not induce any disturbance of the m.c. activity.

In order to eliminate interference from respiration a stable head rest was constructed and the recordings were made from the frontosuperior part of the maxillary sinus, where the mucous membrane lies close to the bony wall. Interference from cardiac activity can be detected by comparing simultaneous ECG and is eliminated by carefully selecting a suitable portion of the sinus mucosa to record from (Fig. 3).

Neither the catheterization of the facial or lingual artery nor the perfusion of the catheter with physiologic saline solution caused any alteration of the m.c. activity in the maxillary sinus.

Experiments with different volumes of physiologic saline solution have shown that bolus doses smaller than 1 ml could be given without disturbing the m.c. activity. Volumes of 1 ml or more had to be given as a slow infusion in order to avoid vascular disturbances. No influence could be detected on the m.c. activity if bolus doses of 0.2 ml, 0.4 ml and 0.6 ml of physiologic saline solution were used. We have therefore standardized the volume of a bolus dose at 0.2 ml and the volume of an infusion at 1 ml.

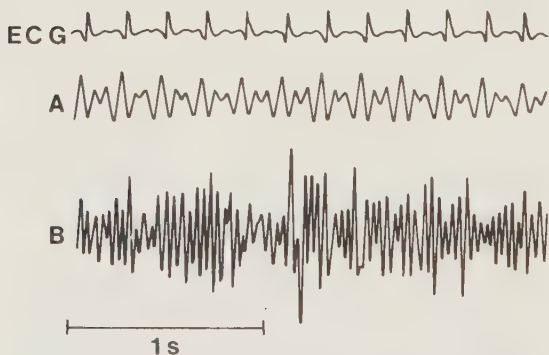


Fig. 3. Simultaneous recordings of ECG and m.c. wave pattern enable a disturbance emanating from the pulse beat to be detected (A). A minor adjustment of the recorded point on the sinus mucosa eliminates this error in recordings (B).

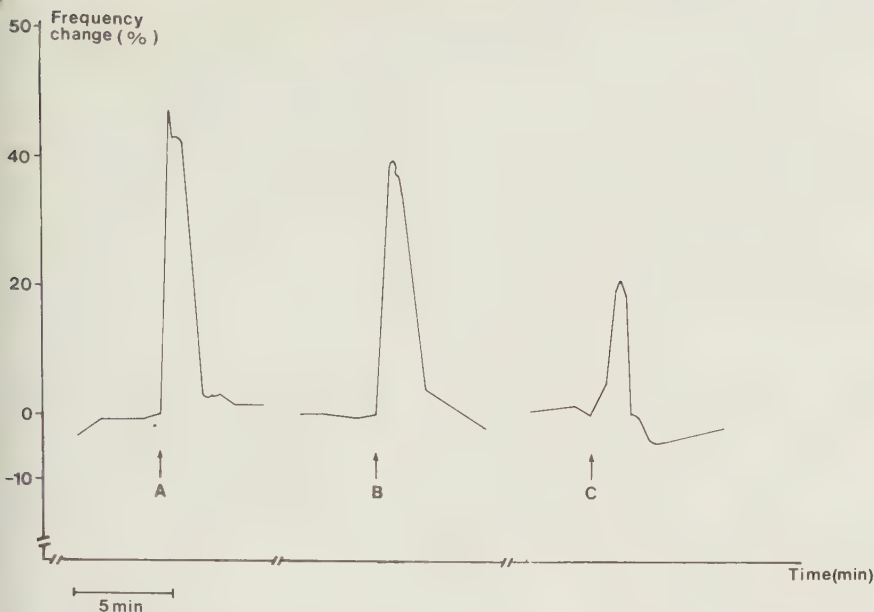


Fig. 4. Effect on m.c. activity after close arterial injections of methacholine $0.8 \mu\text{g/kg}$ in identical bolus doses of 0.2 ml. Duration of injection (A) 3 sec, (B) 10 sec,

and (C) 30 sec: The mean frequency zero level was 1285 waves/min.

Variation of the duration of injection of identical bolus doses in the chosen volume of 0.2 ml showed that prolongation resulted in reduction of the responses. This is exemplified in Fig. 4, where the duration of injection of $0.8 \mu\text{g/kg}$ of methacholine was extended from 3 to 30 sec. We have therefore standardized the duration of injection of a bolus dose at 3 sec and of an infusion at 2 min.

The method was initially used to determine the basal m.c. wave frequency in the maxillary sinus of the anesthetized rabbit. In 66 rabbits, it was found to be 1228 ± 174 (S.D.) waves/min at body temperature $37.0\text{--}38.5^\circ\text{C}$.

In order to test the method the following pharmacological agents were administered intra-arterially either as a 2 min infusion or as a bolus injection.

1. Serotonin (0.0025 , 0.25 and $250 \mu\text{g/kg}$ and min; 6 rabbits) did not show any effect on m.c. activity. The largest dose gave general side effects, and was lethal to most animals tested.

2. ATP (2.5 and $25 \mu\text{g/kg}$ and min; 2 rabbits) also failed to influence m.c. activity.

The higher dose was lethal.

3. Parasympathomimetic substances.

(a) Methacholine infusion (0.25 , 2.5 and $25 \mu\text{g/kg}$ and min; 7 rabbits) and bolus injection (0.4 and $0.8 \mu\text{g/kg}$; 2 rabbits) gave clear and powerful dose-dependent responses in the form of an increased m.c. wave frequency. The 2-min infusion gave an immediate response, after which the frequency declined to normal within 20 min (Fig. 5).

(b) Acetylcholine (0.25 , 2.5 and $25 \mu\text{g/kg}$ and min; 2 rabbits) gave a dose-dependent increase in m.c. wave frequency and the responses were similar to those obtained with methacholine.

(c) Pilocarpine (0.25 , 2.5 and $25 \mu\text{g/kg}$ and min; 2 rabbits) gave a dose-dependent response similar to that of methacholine and acetylcholine.

4. Sympathomimetic substances.

(a) Adrenalin (0.025 , 0.25 , 2.5 and $25 \mu\text{g/kg}$ and min; 2 rabbits) gave a moderate dose-dependent increase of the wave frequency, 20% at most, at higher doses.

(b) Orciprenaline (0.025 , 0.25 and $2.5 \mu\text{g/kg}$

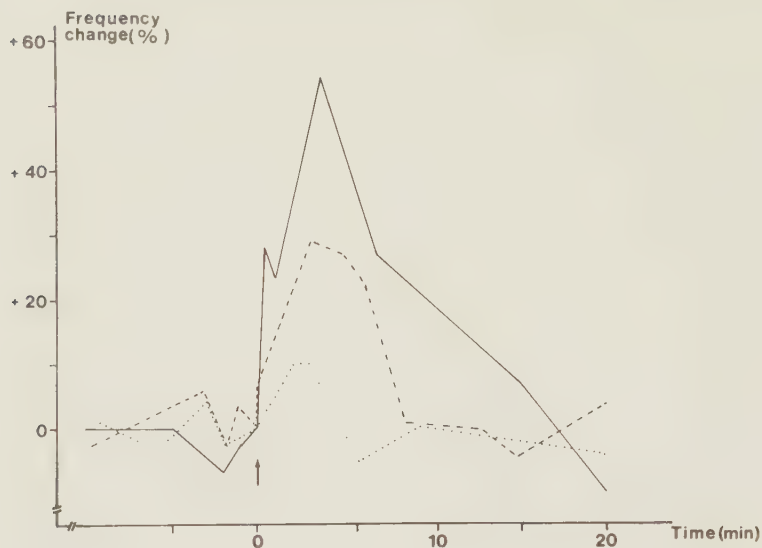


Fig. 5. Effect on m.c. wave frequency after close arterial infusions for 2 min of methacholine at dose rates of 0.25 ($\cdots$), 2.5 ($---$) and 25 ($—$) $\mu\text{g/kg}$ and min. Mean frequency zero level was 1132 waves/min.

and min; 2 rabbits) gave a moderate dose-dependent increase in wave frequency, 25% at most.

(c) Noradrenalin infusion (0.25, 2.5 and 25 $\mu\text{g/kg}$ and min; 2 rabbits) and bolus injection (0.1–20 $\mu\text{g/kg}$; 2 rabbits) influenced the m.c. activity only slightly. In some experiments activity was even reduced.

DISCUSSION

The experiments reported above constitute an *in vivo* animal model for studying the effects of pharmacological substances on m.c. activity in the respiratory tract and where the administered substances reach the observed mucous membrane via the blood vessels (Fig. 1).

The m.c. activity has been registered photoelectrically as previously described and analysed by Mercke et al. (1974a) and later applied to *in vitro* studies of physical factors influencing m.c. activity (Mercke, 1974, 1975; Mercke et al., 1974b; Baldetorp et al., 1976). The photoelectric technique has also been used for *in vivo* studies by Reimer & Toremalm (1978).

The choice of the rabbit as an experimental animal model is based on experience from

earlier *in vitro* experiments on m.c. activity in the rabbit (Mercke et al., 1974a) and from *in vivo* studies on the rabbit maxillary sinus (Reimer & Toremalm, 1978). In the respiratory tract the mucous membrane in the maxillary cavity has been preferred as it can be reached via a major vessel, the maxillary artery (Jeppsson & Olin, 1960) and because vast areas rest against stable bone, enabling disturbances emanating from respiratory activity to be avoided. Sealing the trepanation hole with an antimist window and bonewax restored the sinus ventilation to normal in our test model and protected the mucous membrane from the noxious effects of dry and cold environmental air.

Previous investigations have revealed variations in velocity, frequency, direction and coordination of the m.c. activity between neighbouring areas of the mucous membrane (Hilding, 1957; Iravani, 1973; Reimer & Toremalm, 1978). To ensure that an observed frequency change of the wave pattern is induced by pharmacological exposure it is important to record from a limited and constant area of the mucous membrane. The photoelectric technique fulfils this need and furthermore in contrast to transport and stroboscopy measurements this technique has a minimal burden of

experimental error (Dalhamn & Rylander, 1962; Ballenger & Orr, 1963; Dalhamn, 1970; Toremalm et al., 1975).

In the animal model presented here an improvement is achieved by the urethane anesthesia. Perry (1941) and Boyd (1972) have shown that urethane has no effect on the respiratory tract fluids and is the anesthetic of choice for long-term studies on m.c. activity in the rabbit. Barbiturates, commonly used as general anesthetics in animal models for pharmacological studies, are less suitable because of the relatively short duration of anesthesia, and of their effect on autonomic ganglia (Harvey, 1975) and on m.c. activity (Barski et al., 1959; Sakakura & Proctor, 1972; Iravani, 1975; Landa et al., 1975; Forbes & Gamsu, 1979). This is also illustrated in Fig. 2. The analgetic and long-term hypnotic effects of urethane and the absence of influence on m.c. activity are advantages in these experiments. The urethane anesthesia, the stable cranium holder and the photoelectric technique combine to permit continuous recording of m.c. activity for a long period of time from a constant and limited area of the mucous membrane.

In designing this animal model the *in vivo* technique has been preferred as it permits the test substances to be administered via the blood. The *in vitro* technique necessitates local application of the test substances. The advantages of the *in vivo* technique for m.c. studies have been stated by Ewert (1965).

In order to get reliable and reproducible results and to ensure the animals are in good condition many factors need to be controlled, e.g. respiration, cardiac activity, blood pressure, body temperature and animal state of hydration. Cardiovascular activity can directly disturb the photoelectrical readings of m.c. activity (Fig. 3). This phenomenon was described and analysed by Reimer & Toremalm (1978).

In order to minimize the experimental errors, anesthetic and surgical procedures have been strictly standardized. The same is

true for the selection of animals and the stimulation technique. This latter is illustrated in Fig. 4.

In order not to disturb the normal innervation of the mucous membrane the cannula was advanced through the facial or lingual artery, whereby the sympathetic plexus surrounding the carotid and maxillary artery was left intact (Dahlström & Fuxe, 1965; Malm, 1973).

Apart from the test on anesthetics the method has been used here on arterially administered test substances. The close arterial infusion and injection techniques have the advantage that the test substances can be given in smaller doses compared to *i.v.* administration, whereby general side effects can be reduced. In salivary gland studies, an *i.v.* test substance dose had to be increased 10 to 200 times to obtain the same response as was given by close arterial injection, depending on the substance used (Emmelin et al., 1970; Thulin, 1974, 1976).

To test our method, we have chosen to study some pharmacological substances that have recently (Wanner, 1977) been reported to influence m.c. activity.

Serotonin, proven to have stimulating effects on ciliary activity in mussels (Gosselin, 1966; Aiello & Paparo, 1974) has been ascribed both accelerating and retarding effects on m.c. transport rates and ciliary beat frequency in mammals (Dadaian et al., 1971; Iravani & Melville, 1975). The second test substance used, ATP, has been identified as the energy source for ciliary motility (Vorhaus & Deyrup, 1953; Alexandrov & Arronet, 1956) and investigations have shown improved transport capacity after local application of ATP (Laurenzi et al., 1968; Forrest et al., 1979). Finally, we chose to try the method on some cholinergic and adrenergic substances because there are indications in the literature that these substances also may influence m.c. activity.

When testing our method with serotonin and ATP our results were consistent and indicate that the m.c. wave frequency could not be influenced by these substances, even when they

were given in high enough doses to cause general side effects.

The observed increase of m.c. wave frequency after both cholinergic and adrenergic stimulation is interesting. The immediate response and the dose dependency (Fig. 5) means that these substances may well have a direct effect on the mucous membrane. A difficult question is whether the observed effect was the result of stimulation of the cilia or of altered physical properties of the respiratory tract fluids. Continued physiological studies and additional histologic examinations are necessary to solve these intricate questions.

The method described in this work revealed only small variability of m.c. activity under basal conditions and after stimulation, in contrast to results obtained with transport and clearance methods (Camner et al., 1971; Chopra, 1978).

In conclusion the tests have shown that our test model gives reproducible results and meets the requirements listed in the introduction, and that it can be used as an *in vivo* model to test the effect of pharmacological substances on m.c. activity.

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Ein Tierversuchsmodell zur Bestimmung der Wirksamkeit verschiedener Pharmaka *in vivo* auf die mukoziliäre Aktivität der respiratorischen Schleimhaut in der Kieferhöhle des Kaninchens wird vorgestellt und erläutert. Die Methode erlaubt die Durchführung der Versuche unter physiologischen Bedingungen. Die mukoziliäre Aktivität eines umschriebenen Schleimhautgebietes wurde kontinuierlich während der mehrstündigen Versuchsdauer fotoelektrisch registriert. Experimentell bedingte Stimuli der Schleimhaut wurden weitestgehend vermieden und die physiologische Ventilation der Kieferhöhle beibehalten. Die Applikation der Pharmaka erfolgte intraarteriell (close arterially). Die erhaltenen Resultate werden analysiert und diskutiert. Mögliche Fehlerquellen, insbesondere

Anästhesieverfahren, puls- und atmungsrelatierte Zilienbewegungen, Volumen sowie Injektionsgeschwindigkeit der getesteten Substanz, werden berücksichtigt. Es wurden Serotonin, ATP und verschiedene adrenerge und cholinerge Substanzen untersucht. Unsere Versuchsanordnung lieferte relativ einfach abzulesende und reproduzierbare Resultate.

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EFFECTS OF THE PARASYMPATHOMIMETIC DRUG METHACHOLINE AND ITS ANTAGONIST ATROPINE ON MUCOCILIARY ACTIVITY

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Abstract. The effect on the mucociliary (m.c.) activity of the parasympathomimetic drug methacholine and its antagonist atropine was studied with the aid of a newly developed animal test model, designed for evaluating the effect of pharmacological substances on the m.c. wave frequency. Methacholine i.a. (0.01–2 $\mu\text{g/kg}$) was found to give a dose-dependent acceleration of the m.c. wave frequency, and the threshold dose 0.03 ± 0.02 ($\pm \text{S.D.}$) $\mu\text{g/kg}$ is suggested to be within physiological limits. The anticholinergic drug atropine i.a. (0.05–0.5 mg/kg) did not influence the basal m.c. wave frequency, but a dose of 0.2 mg/kg reduced or abolished the responses to methacholine (0.05–2 $\mu\text{g/kg}$). These results indicate that the basal m.c. activity functions independently of parasympathetic activity in anesthetized animals, that parasympathomimetic drugs influence the m.c. activity and that the effects of these drugs can be reduced or blocked by atropine.

Mucociliary (m.c.) activity is believed to be the major mechanism for eliminating foreign materials from the upper and lower airways (Lucas & Douglas, 1934; Hilding, 1957). A working hypothesis to explain this mechanism is that particulate materials are deposited on a surface viscoelastic mucus sheet (gel layer), and that rhythmic ciliary movements in a watery intercilary fluid (sol layer) propel the covering mucus sheet towards the pharynx, from which it is swallowed (Lucas & Douglas, 1935; Sturgess, 1977).

Of great clinical and theoretical interest are the effects of various drugs on the m.c. activity. Both stimulating and retarding effects have been noted for a variety of pharmacological agents commonly used in clinical practice (Wanner, 1977). The results from different investigations are difficult to compare and are

often conflicting. For instance, parasympathomimetic drugs have been reported to induce such contradictory effects as increased or reduced ciliary beat frequency and accelerated or retarded particle transport rates, while in other investigations no effect at all was observed (see Asmundsson & Kilburn, 1973). Anticholinergic agents have also been ascribed contradictory effects on the m.c. activity, e.g. increased (Chopra, 1978), decreased (Blair & Woods, 1969) or none (Camner et al., 1974).

The differing results reported may to some extent be due to the methods used to study the effects and to differences between species and organs studied. Furthermore, most in vivo investigations include only a few test doses, and those needed to demonstrate the influence have often been unphysiologically large. To our knowledge, no in vivo dose-response relationship has yet been described for parasympathomimetic drug action on m.c. activity. Nor is the site of action, i.e. mainly on the cilia or mainly on the mucus secreting apparatus, of parasympathomimetic agonists and their antagonists clear (Wanner, 1977).

The parasympathetic nervous system is believed to play an important role in the airway defence system (Widdicombe et al., 1962; Camner et al., 1974), but the action of parasympathomimetic agonists and antagonists on the m.c. activity is still not definitely clarified. The present investigation was undertaken to study further the effects of these agonists and antagonists. The method used (Hyb-

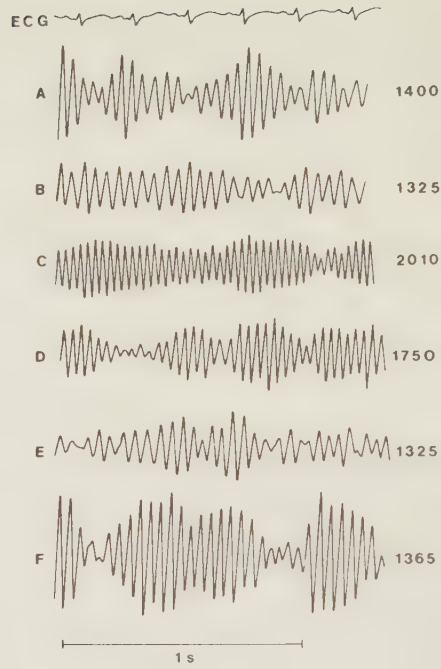
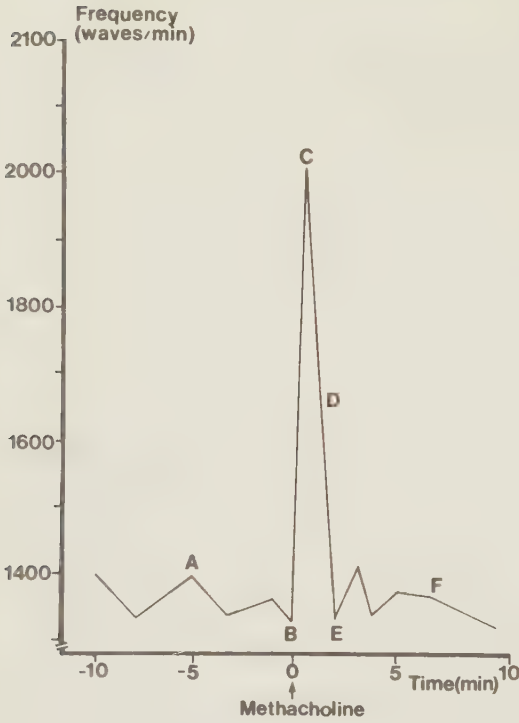


Fig. 1. The effect on the m.c. wave frequency of methacholine $1 \mu\text{g/kg}$ injected close intra-arterially (arrow) as a bolus dose of 0.2 ml during 3 sec. The original record-

ings are exemplified to the right and numbers indicate frequency in waves/min.

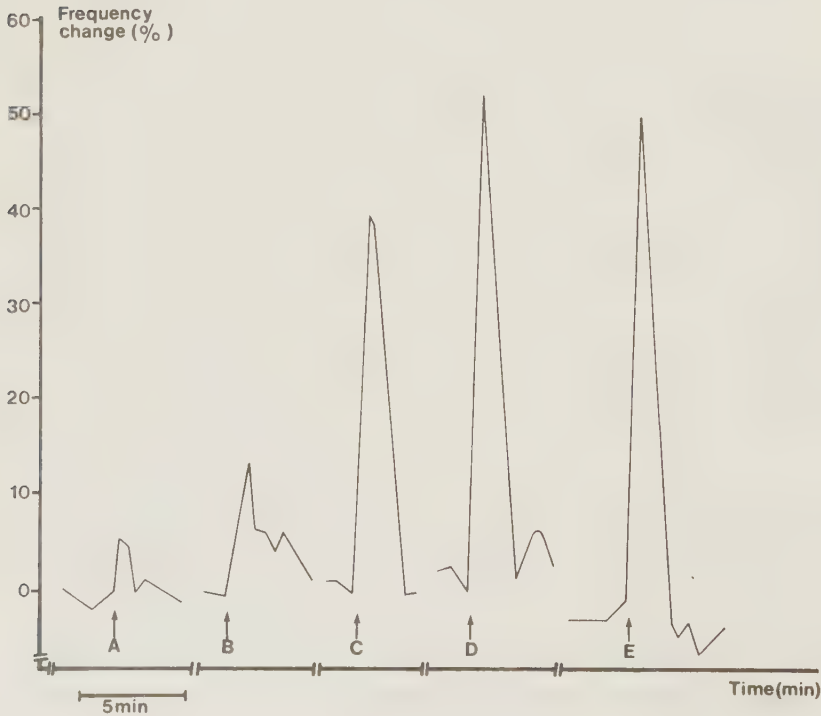


Fig. 2. The typical effect on the m.c. wave frequency in 1 rabbit after close intra-arterial injections of methacholine in the doses: 0.05 (A), 0.1 (B), 0.5 (C), 1 (D) and 2 (E) $\mu\text{g/kg}$. The frequency change is expressed as a percentage of the wave frequency at the instant of injection, and the mean frequency zero level was 1368 ± 46 ($\pm \text{S.D.}$) waves/min.

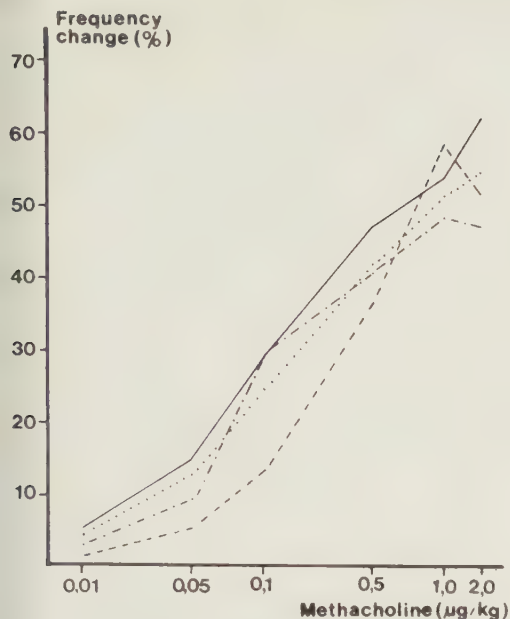


Fig. 3. The effect on the m.c. wave frequency of methacholine i.a. in the dose range 0.01–2 µg/kg when tested in 4 rabbits. The mean frequency zero level for the 23 experiments was 1228 ± 131 ($\pm$ S.D.) waves/min.

binette & Mercke, 1981) has the advantage that the m.c. activity can be studied under conditions that very closely mimic the normal physiological situation. Thus the recording technique is non-invasive and the investigated mucous membrane and its covering mucus sheet are only minimally exposed to environmental disturbances during the experiments.

MATERIAL AND METHODS

The experiments were performed on 39 controlled and healthy male rabbits weighing 1.7–2.5 kg from a white strain. Details of anesthetic and operative techniques were as described previously (Hybbinette & Mercke, 1981). In short the animals were anesthetized with a 2 g/kg i.m. dose of urethane and an extra dose of 0.5 g/kg i.v. was usually given during the ensuing surgical procedure. No additional anesthesia was then needed during the experiments. Regular, spontaneous breathing and elimination of movement by the

animal were deemed to be signs of a satisfactory depth of anesthesia.

The left lingual or facial artery was cannulated for administration of test substances. The cannula was continuously supplied with physiological saline solution at a rate of 5–10 ml/h in order to keep it open and to compensate the animal for dehydration. The maxillary sinus was opened on the same side as the cannulation and the trepanation hole, approximately 2×8 mm in size, was immediately sealed with an antimist window and bone wax. This restored the sinus ventilation to normal and the m.c. activity could be studied through the window. To register the m.c. activity the photoelectric technique described by Mercke et al. (1974a) was used. Variations in surface light reflections brought about by the m.c. activity were followed on an oscilloscope and recorded with an ink writer. The activity was calculated in waves/min, averaged over 20-sec periods. The m.c. wave pattern was recorded continuously during stimulation and the ensuing 3 min, thereafter every min until the wave frequency had returned to prestimulation basal values. The induced frequency changes were expressed as percentages of the basal frequency immediately preceding administration of the test substance.

ECG and rectal temperature were monitored continuously during the experiments, and body temperature was regulated to normal (i.e. 37.0–38.5°C) with the aid of a heating pad.

The following drugs were tested: methacholine chloride (Sigma Chemical Co.) and atropine sulphate (ACO). The drugs were dissolved in physiological saline solution and administered i.a.

For statistical evaluation Student's *t*-test for paired data was used.

Experimental procedure

The m.c. activity was recorded every 5 min until the frequency had been stable for approximately 30 min. The test solutions were then delivered as standardized 3 sec, 0.2 ml retrograde injections through the cannula in the

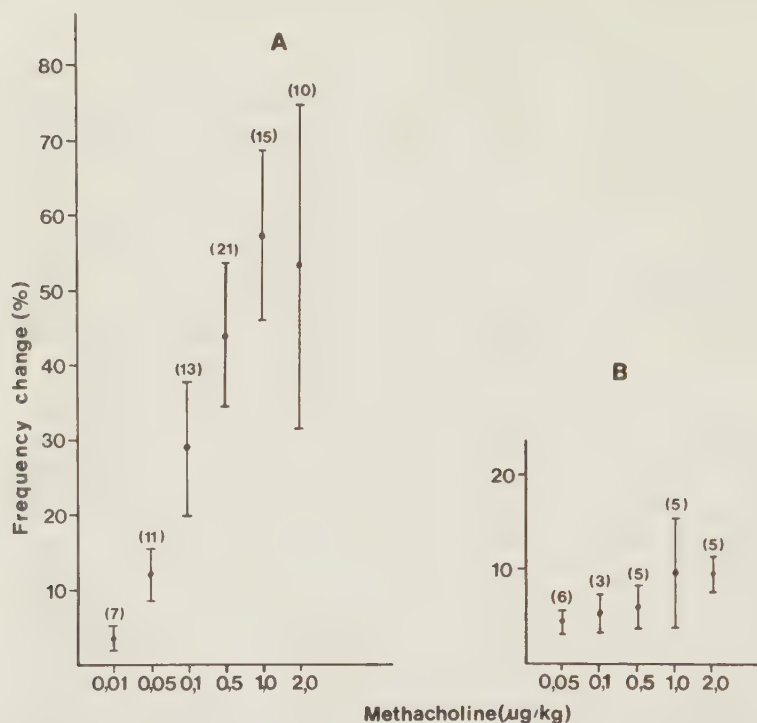


Fig. 4. (A) Summary of the effect on the m.c. wave frequency of methacholine (0.01–2 µg/kg) i.a. (B) The effect on the m.c. wave frequency of methacholine (0.05–2 µg/kg) i.a. given 5 min after atropine (0.2 mg/kg) i.a. Figures in brackets give number of animals examined. Vertical lines give the variation in $\pm$ S.D. of the response

in different animals studied. The mean frequency zero level for the 77 experiments included in (A) was 1193 ± 149 ($\pm$ S.D.) and for the 24 experiments in (B) 1191 ± 220 waves/min, respectively. Note that the entire range of test doses was not given to all animals.

lingual or facial artery. By varying the concentrations of the test substances a wide range of test doses (dose/body weight) were given. Saline injections at the same volume rates

served as controls and did not influence the m.c. activity. The time lapse between renewed injections was approximately 10 min for methacholine and 20 min for atropine.

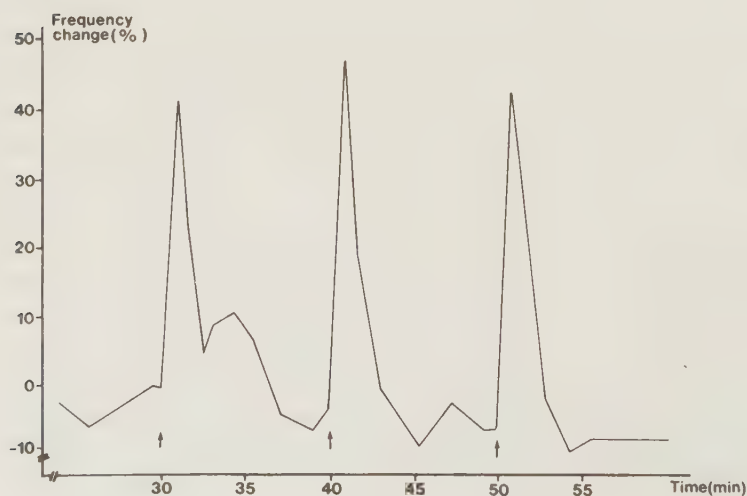


Fig. 5. The effect on the m.c. wave frequency in 1 rabbit of methacholine 0.8 µg/kg when given close intra-arterially 3 times (arrows) at intervals of 10 min. The frequency zero level was 1150 waves/min.

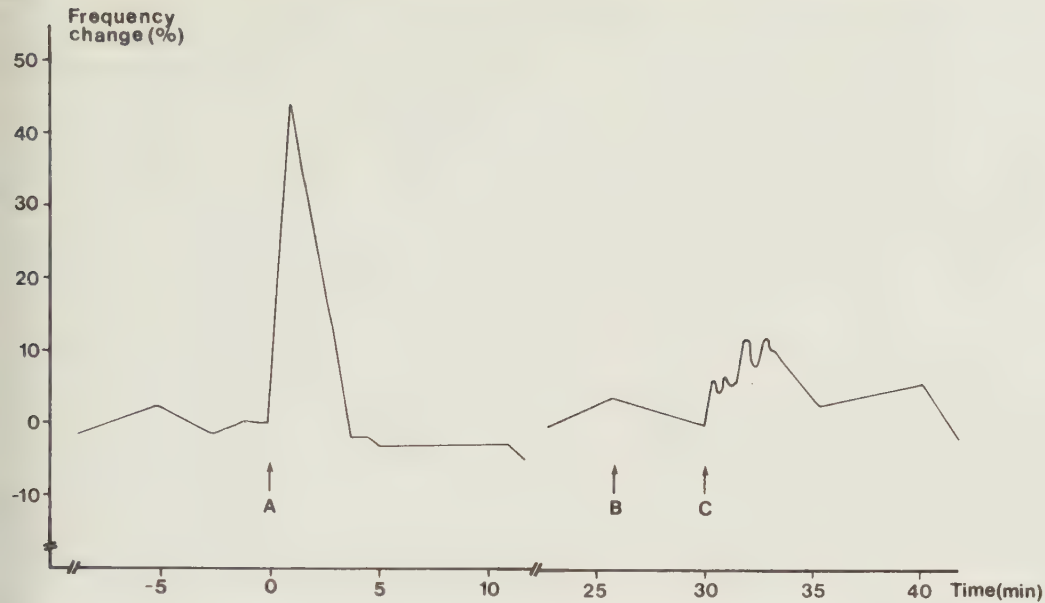


Fig. 6. The effect on the m.c. wave frequency in 1 rabbit of methacholine ($2 \mu\text{g/kg}$) i.a. given 30 min in advance (A) and 5 min after (C) atropine (0.2 mg/kg) i.a. (B). The

frequency zero level at (A) is 1160 and at (C) 1104 waves/min.

To study the effect of atropine on methacholine-induced changes in m.c. wave frequency, atropine was given 5 min in advance of the agonist. Even though some rabbits have a genetically and age-determined circulating atropinase (Bernheim & Bernheim, 1938; Sawin & Glick, 1943) this time interval is regarded as sufficient. Hobbiger & Lessin (1955) reported that about 80% of the blocking effect on muscarinic receptors still remained 10 min after large atropine doses had been given to rabbits known to have high enzymic activity.

RESULTS

Close intra-arterial injections of methacholine increased the m.c. wave frequency. The responses elicited came after a latency of 6–10 sec and remained for 2–5 min, as exemplified in Fig. 1. The calculated threshold dose, defined as the dose that would increase the m.c. wave frequency by 5% or more of the basal level, was found to be 0.03 ± 0.02 ($\pm \text{S.D.}$) $\mu\text{g/kg}$ in 7 rabbits. The increase in m.c. wave

frequency was dose-dependent, which is illustrated in Fig. 2. Fig. 3. shows the dose-effect relationship in the whole range (0.01 – $2 \mu\text{g/kg}$) of methacholine test doses given (4 rabbits). A summary of all results obtained in 21 rabbits when tested with methacholine (0.01 – $2 \mu\text{g/kg}$) is given in Fig. 4A. Analysis of paired data derived from Fig. 4A showed the increase in wave frequency to be significantly higher at $0.1 \mu\text{g/kg}$ than at $0.05 \mu\text{g/kg}$ ($p < 0.01$; $N=7$) and similarly $1 \mu\text{g/kg}$ was found to cause a larger response than that obtained at $0.5 \mu\text{g/kg}$ ($p < 0.01$; $N=7$).

The responses obtained were reproducible irrespective of whether stimulations consisted of increasing or decreasing test doses. Reproducible responses were also obtained if the same dose was given repeatedly (Fig. 5).

Atropine had no effect on the basal m.c. wave frequency, neither when given in single doses of 0.05 – 0.5 mg/kg (35 experiments in 16 rabbits) nor when given as repeated doses of 0.2 mg/kg 3–4 times at intervals of 20 min (24 experiments in 7 rabbits). Injections of atropine (0.2 mg/kg) 5 min in advance of meth-

acholine (0.05–2 $\mu\text{g/kg}$; 24 experiments in 9 rabbits) abolished or greatly reduced the previously found increase in wave frequency caused by methacholine (Fig 4B). One example of this inhibiting effect of atropine on methacholine is given in Fig. 6, where methacholine (2 $\mu\text{g/kg}$) was given 30 min prior to and 5 min after an injection of atropine (0.2 mg/kg).

DISCUSSION

Results of the present investigation demonstrate that the parasympathomimetic drug methacholine (0.01–2 $\mu\text{g/kg}$) accelerates the m.c. wave frequency. While the anticholinergic drug atropine (0.05–0.5 mg/kg) does not itself influence the m.c. activity in the anesthetized rabbit, it does reduce the previously found responses to methacholine (0.05–2 $\mu\text{g/kg}$).

The present investigation has been performed with the aid of the newly developed animal test model described by Hybbinette & Mercke (1981). This model is designed for in vivo analysis of the effects of pharmacological substances on the m.c. activity in the rabbit maxillary sinus. Its advantages are that the m.c. activity is recorded by a non-invasive photoelectric technique and that the test substances reach the investigated mucous membrane in a physiological manner, i.e. via the feeding vessels to the epithelium and not via the covering mucus sheet. This also eliminates mechanical disturbances caused by local application of tracer and test substances. The normal ventilation to the maxillary sinus is retained. This protects the sinus mucous membrane from the effects of variations in the temperature and humidity of the air, environmental factors which have been found in previous investigations to influence the m.c. activity (Mercke et al., 1974*b*; Mercke, 1975).

While developing the test model used (Hybbinette & Mercke, 1981) preliminary data were obtained which indicate that the parasympathomimetic substances methacholine, acetyl-

choline and pilocarpine all have an accelerating effect on the m.c. wave frequency. Methacholine, a β -methyl ester of acetylcholine, was selected for the present investigation, as it acts mainly on postganglionic receptors and also because it is less susceptible to cholinesterase than acetylcholine (Koelle, 1975).

The present results were that methacholine given close intra-arterially in the dose range 0.01–2 $\mu\text{g/kg}$ increased the m.c. wave frequency and that this increase was dose-dependent. Our results contradict proposals by some early investigators that m.c. transport activity may function independently of nervous and humoral control (Hill, 1928; Lucas & Douglas, 1935) or even that parasympathomimetic drugs decrease m.c. transport rates (McDonald et al., 1928; King & Viires, 1979). On the contrary, our findings are in accordance with several other investigations showing accelerated m.c. transport rates after parasympathomimetic drug stimulations in vitro (Umeda, 1929; Kordik et al., 1952; Iravani & Melville, 1975) and in vivo (Albert et al., 1973; Camner et al., 1974; Wanner et al., 1975). Whereas the conclusions from these in vivo investigations are based on results obtained from only few single doses our own study is comprehensive with series of injections (6 different doses and 21 animals; see Figs. 3, 4). We are thus confident that there is a clear dose-effect relationship and that nervous control could be involved in m.c. activity.

It has, however, been proposed (Gosselin, 1966) that acetylcholine does not play any physiological role in the control of cilia in mammals, because the reported effects of this neurotransmitter are small and the doses required rather large. The 0.03 ± 0.02 $\mu\text{g/kg}$ threshold dose of methacholine found in our study can anyhow be regarded as being within physiological limits, since it corresponds well with threshold doses that evoke salivary flow in rat salivary glands, which are parasympathetically innervated (Thulin, 1976).

In our investigation atropine (0.2 mg/kg)

did not alter the basal m.c. wave frequency in the rabbit maxillary sinus, but it reduced or inhibited the previously found and expected response to methacholine ($0.05\text{--}2\text{ }\mu\text{g/kg}$; Figs. 4, 6). These results indicate that there is no basal or reflex cholinergic activity influencing the m.c. wave frequency in the anesthetized rabbit and that anticholinergic substances block the action of parasympathomimetic drugs on the m.c. activity.

Our results concerning this blocking effect of anticholinergic drugs are in agreement with an early *in vitro* investigation by Plattner & Hou (1931). Our other finding, that these drugs do not themselves influence basal m.c. activity, has more recently also been demonstrated by Camner et al. (1974) and Lopez-Vidriero et al. (1975). In other *in vitro* and *in vivo* investigations atropine was found to increase (Chopra, 1978) or to decrease (Gosselin, 1966; Annis et al., 1976; Giordano et al., 1978) m.c. transport rates, and also to retard ciliary beat frequency (Corssen & Allen, 1959; Blair & Woods, 1969). These differences in reported data can be explained by variations in materials and experimental methods, and by basal or reflex cholinergic activity. As atropine in our study did not influence the basal m.c. activity, but reduced the effects of methacholine, we conclude that anticholinergic substances only influence the m.c. activity when this is already accelerated by cholinergic stimulation.

It has been proposed that parasympathomimetic drugs affect the m.c. activity either by acting on the cilia (Burn, 1954; Corssen & Allen, 1959) or on the mucus-secreting apparatus (Bang et al., 1966; Sakakura & Proctor, 1972; King & Viires, 1979). Cholinergic substances have been shown to alter the quantity of mucus (Florey et al., 1932; Nadel & Davis, 1978) and to alter its physical properties (King & Viires, 1979). Davis et al. (1975) have recently proposed that cholinergic substances give rise to a watery secretion mediated by a net ion flux across the mucous membrane. In our experiments methacholine ($0.01\text{--}2\text{ }\mu\text{g/kg}$) gave rise to an immediate dose-depen-

dent increase in m.c. wave frequency which returned to the prestimulation value within 2–5 min (Figs. 1, 2). Reproducible increases were also obtained within 10 min with repeated doses of methacholine $0.8\text{ }\mu\text{g/kg}$ (Fig. 5). This might be the result of an influence of the drug on the mucus-secreting apparatus, but it has not previously been demonstrated that cholinergically induced changes in mucus volume or physical properties have such short time limits as the effects on the m.c. wave frequency found in our study (cf. Reasor et al., 1978; King & Viires, 1979). For this reason we are inclined to believe that the increase in m.c. wave frequency following methacholine stimulation is mainly due to a drug action on the cilia and not on the mucus-secreting apparatus.

Even though the results from animal experiments cannot be transferred directly to clinical practice, our finding that parasympathomimetic drugs accelerate m.c. activity is supported by clinical observations in man showing increased m.c. clearance rates following injections of parasympathomimetic drugs (Albert et al., 1973; Camner et al., 1974). Although not proven, stimulation of parasympathetic vagal nerve fibres would also be expected to increase m.c. activity (cf. Camner et al., 1974). Similarly other protective mechanisms of the human lung, e.g. coughing and bronchoconstriction, are believed to be facilitated or mediated by reflexly evoked activity in parasympathetic vagal nerve fibres (Widdicombe et al., 1962; Albert et al., 1973). Hence it cannot be excluded that anticholinergic drugs have adverse effects not only on parasympathomimetically induced improvements of the m.c. activity, but also on other airway defence mechanisms in man.

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ZUSAMMENFASSUNG

Die mukoziliäre Aktivität unter Einfluss des Parasympathomimetikums Metacholin und seines Antagonisten Atropin wurde an einem neu entwickelten TierversuchsmodeLL untersucht, das die Auswertung pharmakologischer Wirkung auf die mukoziliäre Schlagfrequenz zulässt.

Metacholin in einer Dosis von 0,01–2 µg/kg bewirkte eine Beschleunigung der mukoziliären Schlagfrequenz. Der Schwellenwert von $0,03 \pm 0,02$ ($\pm$ S.D.) µg/kg scheint innerhalb physiologischer Grenzen zu liegen.

Das Parasympatholytikum Atropin (0,05–0,5 mg/kg) zeigte keinen Effekt auf die basale mukoziliäre Aktivität, jedoch reduzierte oder neutralisierte es in einer Dosis von 0,2 mg/kg die Wirkung von Metacholin (0,05–2 µg/kg).

Diese Resultate lassen den Schluss zu, dass die basale Aktivität nicht vom parasympathischen Tonus abhängt. Die Steigerung der parasympathischen Reize zieht allerdings unter physiologischen Bedingungen eine erhöhte mukoziliäre Aktivität nach sich. Die Ergebnisse indizieren darüberhinaus ungünstige Nebenwirkungen anticholinerg wirkender Pharmaka auf die mukoziliäre Transportkapazität.

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EFFECTS OF SYMPATHOMIMETIC AGONISTS AND ANTAGONISTS ON MUCOCILIARY ACTIVITY

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Abstract. The effect on the mucociliary (m.c.) wave frequency of sympathomimetic agonists and antagonists was studied in the maxillary sinus in anesthetized rabbits. The non-selective β -adrenoceptor agonist isoprenaline (0.005–10 $\mu\text{g/kg}$, i.a.) and the selective β_2 -adrenoceptor agonist salbutamol (0.01–10 $\mu\text{g/kg}$, i.a.) induced a dose-dependent acceleration of the m.c. wave frequency, whereas the β_1 -adrenoceptor agonist prenalterol (1–200 $\mu\text{g/kg}$, i.a.) did not influence the basal m.c. activity. The selective α_1 -adrenoceptor agonist phenylephrine (0.01–20 $\mu\text{g/kg}$, i.a.) and the selective α_2 -adrenoceptor agonist oxymetazoline (0.001–1 $\mu\text{g/kg}$, i.a.) both induced a dose-dependent retardation of the m.c. wave frequency. The non-selective β -adrenoceptor antagonist propranolol (2–200 $\mu\text{g/kg}$, i.a., and 1 mg/kg, i.v.) and the non-selective α -adrenoceptor antagonist phentolamine (0.1–1000 $\mu\text{g/kg}$, i.a.) had no influence on the basal m.c. wave frequency. Propranolol (1–2 mg/kg, i.v.) reduced the effect of isoprenaline and salbutamol (both in the dose range of 0.01–10 $\mu\text{g/kg}$, i.a.) and similarly phentolamine (0.2 and 1 mg/kg, i.a.) reduced the effect of oxymetazoline (0.01–10 $\mu\text{g/kg}$, i.a.).

It was concluded that during basal conditions in the anesthetized rabbit the m.c. activity functions independently of sympathetic activity and that sympathomimetic agonists acting on β_2 -adrenoceptors accelerate the wave frequency, whereas sympathomimetic agonists acting on α_1 and α_2 -adrenoceptors have a retarding effect, all in a dose-dependent manner.

Drugs acting on the autonomous nervous system are commonly used in the treatment of airway diseases. However, several questions regarding the effects of sympathomimetic and parasympathomimetic substances on mucociliary (m.c.) activity still remain unsolved (Wanner, 1977). It is generally accepted that m.c. activity is an important defence mechanism as is illustrated by the increased incidence of chronic or recurrent infections in the upper and lower airways in persons with non-functioning cilia (Afzelius, 1979). It is

therefore important to elucidate the effects of such drugs on m.c. activity.

In a previous investigation on rabbit (Hybbinette & Mercke, 1981b) the parasympathomimetic agonist methacholine (0.01–2 $\mu\text{g/kg}$, i.a.) was found to give a dose-dependent increase of the m.c. activity. In contrast, atropine (0.05–0.5 mg/kg, i.a.) did not influence m.c. activity. However, in a dose of 0.2 mg/kg i.a. atropine inhibited the effect of methacholine.

Although sympathomimetic substances have been extensively studied (Innes & Nickerson, 1975), information about their effect on m.c. activity is sparse and often conflicting (Proctor & Adams, 1976). Of clinical interest is also the suggestion that some sympathomimetic agonists may reduce m.c. transport capacity and thus play a role in the etiology of diseases in the upper airways (Peerless & Noiman, 1980).

There are two groups of receptors with which sympathomimetic substances can react to elicit a response in the effector cells. Ahlquist (1948) classified these receptor sites as α and β on the basis of their responses to a series of sympathomimetic amines. Lands et al. (1967) showed that the β -adrenoceptor population can be further divided into β_1 and β_2 -adrenoceptors (see Hedberg, 1980), and similarly the α -adrenoceptors can be divided into two groups, α_1 and α_2 (Langer, 1974; see Wikberg, 1979; Andersson, 1980).

It has been suggested that the distribution of β_1 and β_2 -adrenoceptors is uneven in dif-

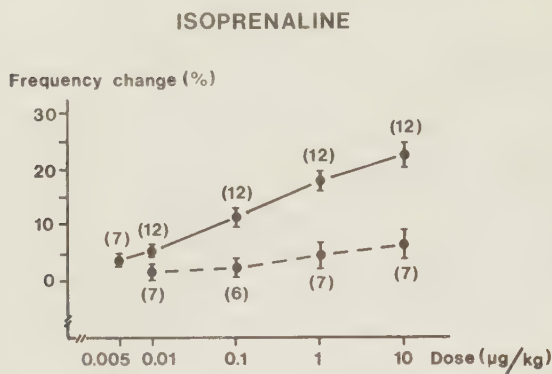


Fig. 1. Effect (mean $\pm$ S.E.M.) of various doses of isoprenaline on m.c. wave frequency before (●—●) and after propranolol 1–2 mg/kg i.v. (○---○). Ordinate: increase in wave frequency as percentage of frequency at instant of injection (zero level); abscissa: doses of the drug on a logarithmic scale. The frequency zero level was $1\,228 \pm 208$ waves/min (mean $\pm$ S.D.) before and $1\,183 \pm 135$ waves/min after propranolol. Numbers in parentheses indicate number of animals examined.

ferent species and in different airway regions (Reid & Jones, 1979). In addition, β_2 -adrenoceptor agonists have been shown to influence m.c. activity in various degrees at different levels of the pulmonary airways (Foster et al., 1980). However, neither the effect of selective β_1 -adrenoceptor agonists on m.c. activity generally nor the specific effect of selective β_1 and β_2 -adrenoceptor agonists on m.c. activity in the upper airways are very well known. Knowledge is also lacking as to whether β -adrenoceptor antagonists by themselves influence in vivo m.c. activity and whether such antagonists influence any effect of β -adrenoceptor agonists on m.c. activity.

The effect of α -adrenoceptor agonists on m.c. activity is also unclear (Peerless & Noiman, 1980). These substances have been ascribed such contradictory effects as increased (Saketkhoo et al., 1978), decreased (Simon et al., 1977), and unaffected (Petruson, 1981) m.c. transport rates in the nasal airways of human volunteers.

Although several sympathomimetic substances are commonly employed in clinical practice, their effect on m.c. activity is not yet clarified. The aims of the present investiga-

tion are therefore (1) to study the effect on the m.c. activity of sympathomimetic agonists with selectivity to different groups of adrenoceptors, (2) to find out whether antagonists acting on β and α -adrenoceptor sites have any influence on their own, and (3) to see if such antagonists influence the respective effects of β and α -adrenoceptor agonists. The present investigation has been performed with the aid of a newly developed animal test model (Hybbinette & Mercke, 1981a) designed for evaluating the effect of pharmacological substances on the m.c. wave frequency in the maxillary sinus in anesthetized rabbits.

MATERIAL AND METHODS

Forty-three healthy male rabbits, 1.6–2.4 kg, from a white strain, were used. Details of anesthetic and operative techniques were as described previously (Hybbinette & Mercke, 1981a). The animals were given urethane (2–2.5 g/kg) and the left facial or lingual artery was retrogradely cannulated. This cannula was used for administering the test substances. In order to keep it open and to compensate the animals for dehydration it was continuously perfused with physiologic saline solution, 5–10 ml/h. The maxillary sinus was opened on the same side as the cannulation. The trepanation hole, approximately 2×8 mm in size, was immediately sealed with an antimist window and bone wax, which restored the sinus ventilation to normal. The m.c. activity in the fronto-superior part of the maxillary sinus was observed through the window. A cold light beam was directed into the sinus and variations in the surface reflections, brought about by the m.c. activity, were registered by a photoelectric technique (Mercke et al., 1974). The reflections were monitored on an oscilloscope and recorded with an ink writer. The wave pattern was recorded continuously during drug stimulations and at intervals of 1–5 min otherwise. The wave frequency was averaged over 20 sec recordings and expressed in waves/min. Changes in m.c. wave

SALBUTAMOL

Frequency change (%)

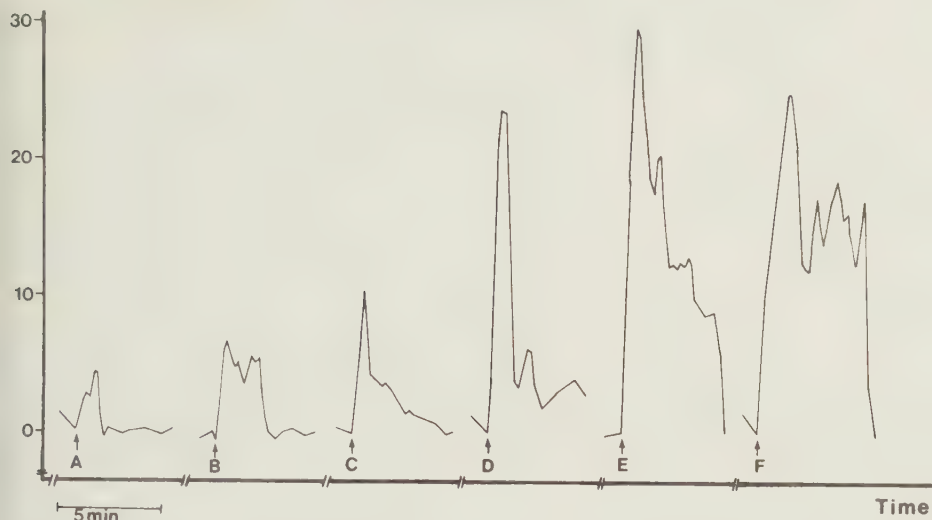


Fig. 2. Effect on m.c. wave frequency in one rabbit of injections of salbutamol 0.01 (A), 0.05 (B), 0.1 (C), 1 (D), 10 (E), and 20 (F) $\mu\text{g/kg}$. Ordinate as in Fig. 1; abscissa:

time (min). The mean frequency zero level was 1296 ± 26 ($\pm \text{S.D.}$) waves/min.

frequency caused by test substances were expressed as percentages of the mean basal m.c. wave frequency prevailing immediately before stimulation (zero level).

During the experiments ECG and rectal temperature were monitored continuously and body temperature was adjusted to normal (i.e. $37.0\text{--}38.5^\circ\text{C}$) with the aid of heating pad.

The following sympathomimetic drugs were tested: β -adrenoceptor agonists: prenalterol hydrochloride (Hässle), isoprenaline sulphate (Riker) and salbutamol sulphate (Glaxo); β -adrenoceptor antagonist: propranolol hydrochloride (ICI); α -adrenoceptor agonists: phenylephrine hydrochloride (Boehringer Ingelheim) and oxymetazoline hydrochloride (Merck); α -adrenoceptor antagonist: phentolamine mesylate (CIBA).

To check the functional state of the test model the parasympathomimetic agonist methacholine chloride (Sigma) was used.

The drugs were dissolved in physiological saline solution and administered i.a. through the cannula as standardized 0.2 ml, 3 sec bolus injections. Different doses were given by

varying the concentrations of the test solutions. Injections of physiologic saline at the same volume rates served as controls. The following exceptions to this standardized procedure were made:

(1) Oxymetazoline and phenylephrine were given as injections of 0.2 ml during 10 sec instead of 3 sec; in animals treated with these substances an injection of physiological saline solution 0.2 ml i.a. during 3 sec occasionally gave rise to a moderate increase of the m.c. wave frequency (at most $+7.5\%$); however, this did not occur after a 10 sec injection.

(2) Propranolol, when given in a larger dose than 0.2 mg/kg, was given i.v. in an ear vein instead of i.a., as such large doses had to be dissolved in volumes exceeding 0.2 ml; previous experience (Hybbinette & Mercke, 1981a) showed that i.a. bolus injections larger than 1 ml should be avoided.

The time interval between repeated injections of the test substances was 10–20 min. In the studies of the blocking effect of propranolol and phentolamine, the time interval between the administration of these antago-

SALBUTAMOL

Frequency change (%)

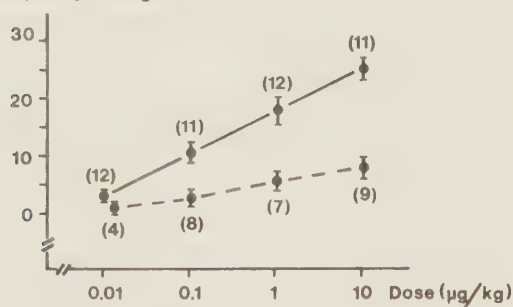


Fig. 3. Effect (mean $\pm$ S.E.M.) of various doses of salbutamol on m.c. wave frequency before (●—●) and after propranolol 1–2 mg/kg i.v. (●---●). Ordinate, abscissa and numbers in parentheses are as in Fig. 1. Frequency zero level was 1185 ± 201 waves/min (mean $\pm$ S.D.) before and 1220 ± 156 waves/min after propranolol.

nists and the ensuing injections of the β and α -adrenoceptor agonists was 10–60 min and 30–40 sec, respectively. This difference in time interval was due to the known differences in duration of the blocking effect between propranolol and phentolamine (Harvey & Nickerson, 1953; Asking & Gjørstrup, 1980).

The threshold dose, defined as the dose of a substance that induced a 5% change in wave frequency, was found by interpolation from both paired and unpaired data.

For statistical evaluation Student's *t*-test for paired and unpaired data and tests of linear contrast were performed (Dunn & Clark, 1974). *P*-values smaller than 0.05 were considered significant.

RESULTS

Adrenoceptor antagonists

(1) The non-selective β -adrenoceptor blocking substance propranolol, when given in the dose range of 2–200 μ g/kg i.a. (13 rabbits) or 1 mg/kg i.v. (16 rabbits) did not influence the basal m.c. wave frequency.

(2) The non-selective α -adrenoceptor blocking substance phentolamine, when given in the dose range of 0.1–1000 μ g/kg i.a. (9 rabbits)

did not influence the basal m.c. wave frequency.

 β -adrenoceptor agonists

(1) Prenalterol, which mainly acts on β_1 -adrenoceptors (Hedberg et al., 1980), was given in the dose range of 1–200 μ g/kg (6 rabbits). It did not influence the basal m.c. wave frequency, neither immediately after injection nor within 2 h.

(2) Isoprenaline, a non-selective β -adrenoceptor agonist, accelerated the m.c. wave frequency. When given in the dose range of 0.005–10 μ g/kg (12 rabbits) this increase was found to be dose-dependent ($p < 0.001$ by linear contrast; Fig. 1). The response came after a latency of 30–40 sec and lasted for 2–5 min. Maximum effect was obtained with the dose of 10 μ g/kg, which accelerated the m.c. wave frequency by $22.4 \pm 5.3\%$ (mean $\pm$ S.D.). Larger doses did not increase the m.c. activity further. The threshold dose derived from the paired data in Fig. 1 was 0.01 μ g/kg (range 0.005–0.05 μ g/kg). Propranolol 1–2 mg/kg i.v. significantly reduced the acceleration of the m.c. wave frequency evoked by isoprenaline 0.1–10 μ g/kg i.a. ($p < 0.01$, by analysis of unpaired data; 7 rabbits), whereas the response obtained from isoprenaline 0.01 μ g/kg was not significantly reduced by propranolol.

(3) Salbutamol, which mainly acts on β_2 -adrenoceptors, accelerated the m.c. wave frequency when given in the dose range of 0.01–20 μ g/kg. One example is illustrated in Fig. 2. The response came after a latency of 30–40 sec and lasted for 2–5 min. There was a dose-dependent increase of the wave frequency in response to increasing doses of salbutamol, 0.01–10 μ g/kg ($p < 0.001$ by linear contrast; 12 rabbits; Fig. 3). The threshold dose derived from the paired data in Fig. 3 was 0.08 μ g/kg (range 0.05–0.1 μ g/kg). The maximum effect was obtained with a dose of 10 μ g/kg, which accelerated the m.c. wave frequency by $24.7 \pm 6.3\%$ (mean $\pm$ S.D.). Propranolol 1–2 mg/kg i.v. significantly reduced the increase in wave

PHENYLEPHRINE

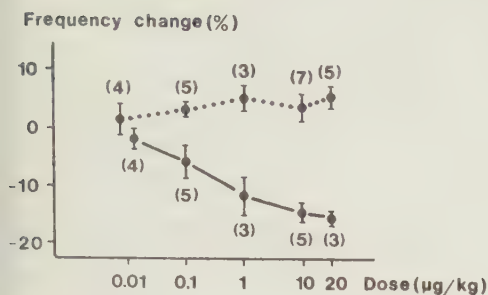


Fig. 4. Effect (mean $\pm$ S.E.M.) of various doses of phenylephrine on m.c. wave frequency when given for the first time or at intervals exceeding 60 min (●—●), and when given in cumulative doses at intervals of 10–20 min, starting with 0.01 μ g/kg (●····●). Ordinate, abscissa and numbers in parentheses are as in Fig. 1. Mean frequency zero level for all 39 experiments was 1236 ± 167 ($\pm$ S.D.) waves/min.

frequency evoked by salbutamol 0.01–10 μ g/kg i.a. ($p < 0.01$, by analysis of unpaired data; 9 rabbits), except for the dose of 0.01 μ g/kg (Fig. 3).

α -adrenoceptor agonists

(1) Phenylephrine, which acts mainly on α_1 -adrenoceptors (Wikberg, 1978), was given in the dose range 0.01–20 μ g/kg (11 rabbits). The effect on the m.c. wave frequency was found to depend on the injection procedure during the experiments. When given for the first time or when given repeatedly at time intervals exceeding 60 min, phenylephrine 0.01–20 μ g/kg retarded the m.c. wave frequency (Fig. 4). This deceleration came after a latency of 30–60 sec and stayed for 5–10 min. The reduction of the wave frequency was dose-dependent, a dose of 10 μ g/kg retarding the frequency significantly more than 0.1 μ g/kg ($p < 0.001$, by analysis of unpaired data). The threshold dose derived from the unpaired data in Fig. 4 was 0.08 μ g/kg (range 0.01–1 μ g/kg). Cumulative doses of phenylephrine given within 10–20 min, starting with sub-threshold doses, produced only an insignificant change in wave frequency, even with a dose of 20

OXYMETAZOLINE

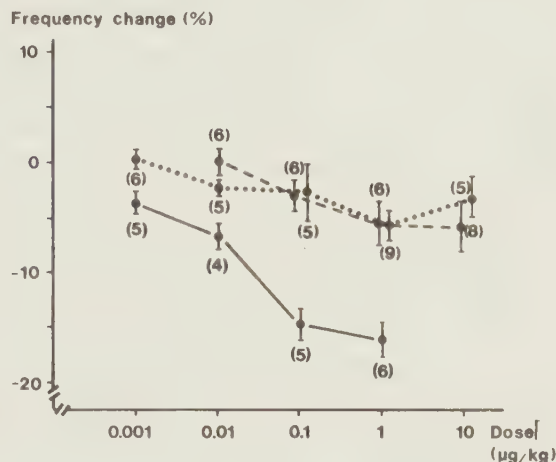


Fig. 5. Effect (mean $\pm$ S.E.M.) of various doses of oxymetazoline on m.c. wave frequency (●—●), when given after phentolamine 0.2 and 1 mg/kg i.a. (●---●) and when given in cumulative doses at intervals of 10–20 min, starting with 0.001 μ g/kg (●····●). Ordinate, abscissa and numbers in parentheses are as in Fig. 1. Mean frequency zero level for all 76 experiments was 1327 ± 223 ($\pm$ S.D.) waves/min.

μ g/kg (Fig. 4). The difference in response to phenylephrine when given in cumulative doses versus isolated single doses was significant for doses of 0.1 and 10 μ g/kg ($p < 0.01$, by analysis of unpaired data). This difference was not significant, however, for a dose of 0.01 μ g/kg. Statistical evaluation was inappropriate for doses of 1 and 20 μ g/kg owing to the small number of observations.

(2) Oxymetazoline, which acts mainly on α_2 -adrenoceptors (Wikberg, 1978) was tested in the dose range of 0.001–10 μ g/kg (18 rabbits). The action on the m.c. wave frequency was found to depend on the injection procedure. When given for the first time or when the time interval between repeated injections exceeded 60–90 min it was found to retard the m.c. wave frequency. When given in the dose range of 0.001–1 μ g/kg (17 rabbits), the decrease in wave frequency was dose-dependent ($p < 0.01$, by analysis of linear contrast; Fig. 5). The threshold dose derived from the unpaired data in Fig. 5 was 0.005 μ g/kg (range

Frequency (waves/min)

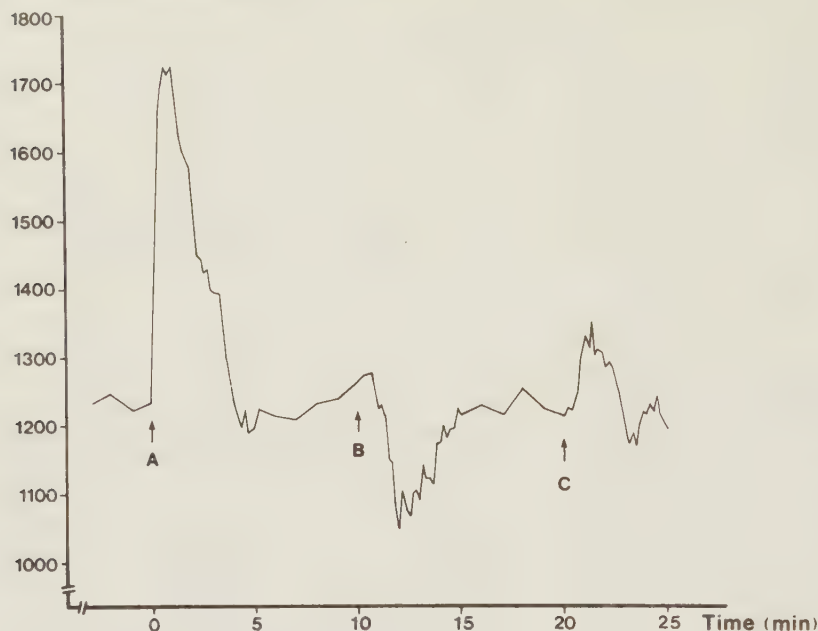


Fig. 6. Effect of methacholine 1 $\mu\text{g/kg}$ on m.c. wave frequency in one rabbit 10 min in advance (arrow A) and 10 min after (C) an injection of oxymetazoline 0.1 $\mu\text{g/kg}$ (B).

0.001–0.01 $\mu\text{g/kg}$). The retardation of the wave frequency was observed within 30–60 sec after injection and the effect lasted for 5–15 min. One example is given in Fig. 6 (see at arrow B), showing the effect on the m.c. wave frequency of an injection of oxymetazoline 0.1 $\mu\text{g/kg}$. Cumulative doses of oxymetazoline within 10–20 min, starting with sub-threshold doses, produced only an insignificant decrease in wave frequency, even with a dose of 10 $\mu\text{g/kg}$ (Fig. 5). Phentolamine 0.2 and 1 mg/kg abolished or strongly reduced the retarding effect of oxymetazoline 0.01–10 $\mu\text{g/kg}$ ($p < 0.02$ at 0.01 $\mu\text{g/kg}$ and $p < 0.001$ at 0.1–1 $\mu\text{g/kg}$, by analysis of unpaired data; 7 rabbits). The response to oxymetazoline (0.01–10 $\mu\text{g/kg}$) in cumulative doses versus after phentolamine (0.2–1 mg/kg) was not significant.

Methacholine 0.5–1 $\mu\text{g/kg}$ was given to check the functional state of the test model. The responsiveness to methacholine was not affected, except in animals already treated with α -adrenoceptor agonists where the effect of methacholine was reduced. When methacholine was given in a dose of 0.5–1 $\mu\text{g/kg}$

to animals treated with oxymetazoline 0.1–1 $\mu\text{g/kg}$, this reduction was significant ($p < 0.001$, by analysis of paired data; 6 rabbits; Fig. 6).

DISCUSSION

The present investigation indicates that the m.c. wave frequency in the maxillary sinus in healthy rabbits is (1) accelerated by the non-selective β -adrenoceptor agonist isoprenaline and by the β_2 -adrenoceptor agonist salbutamol, (2) retarded by the α_1 -adrenoceptor agonist phenylephrine and by the α_2 -adrenoceptor agonist oxymetazoline, (3) unaffected by the β_1 -adrenoceptor agonist prenalterol, (4) unaffected by the α and β -adrenoceptor antagonists phentolamine and propranolol *per se*, but that these substances reduce the magnitude of the effects of α and β -adrenoceptor agonists, respectively. In addition it is shown that (5) the accelerating effect of the parasympathomimetic agonist methacholine is reduced by the α_2 -adrenoceptor agonist oxymetazoline.

The selective β_2 -adrenoceptor agonist sal-

butamol and the non-selective β -adrenoceptor agonist isoprenaline both accelerated the m.c. wave frequency in a dose-dependent manner (Figs. 1, 2, 3), whereas prenalterol, mainly acting on β_1 -adrenoceptors, did not influence the basal m.c. wave frequency. The magnitude of this accelerating effect was markedly reduced by propranolol (Figs. 1, 3). Thus it appears that β_2 -adrenoceptors are present in the regulation of the m.c. wave frequency in the rabbit maxillary sinus.

The reason for the acceleration of the m.c. wave frequency after administration of β_2 -adrenoceptor agonists cannot be determined from our data. As compared with the action of the parasympathomimetic substance methacholine (Hybbinette & Mercke, 1981*b*) isoprenaline and salbutamol were less effective in their accelerating effect on m.c. wave frequency (at most 22.4–24.6% versus 57.7%) and also the responses came after a longer latency (30–40 sec versus 6–10 sec). Whether these differences can be attributed to different effector cells for the two kinds of agonists cannot be evaluated, since our method only measures changes in the m.c. wave frequency (cf. Staub, 1975; Foster et al., 1976).

The action of β_2 -adrenoceptor agonists on m.c. activity in the paranasal sinuses does not seem to be investigated before, but our results are in accordance with several previous investigations in healthy pulmonary airways *in vitro* (Iravani, 1972) and *in vivo* (Camner et al., 1976; Foster et al., 1976, 1980). In contrast Santa Cruz et al. (1974) and Sackner et al. (1976) reported that β_2 -adrenoceptor agonists accelerated m.c. activity only in diseased pulmonary airways and not in healthy, where the activity was assumed to be optimal. However, this optimal activity seems to be related only to the m.c. transport and clearance capacity in the pulmonary airways, which they measured, and not to the sinus m.c. wave frequency in healthy rabbits, which was investigated in our study.

The present results indicate that phenylephrine (which mainly acts on α_1 -adrenocep-

tors) and oxymetazoline (which mainly acts on α_2 -adrenoceptors) both retard the m.c. wave frequency (Figs. 4, 5), and that phentolamine reduces the magnitude of the decelerating effect of oxymetazoline (Fig. 5). Furthermore, in the control of the functional state of the test model oxymetazoline was found to reduce the accelerating effect of the parasympathomimetic agonist methacholine when given via the feeding vessel to the mucous membrane (Fig. 6). These findings indicate that both α_1 and α_2 post-junctional adrenoceptors participate in regulating the m.c. wave frequency in the paranasal sinuses.

The retarded m.c. wave frequency following administration of these α -adrenoceptor agonists, as well as the reduced effect of these drugs when given *i.a.* in cumulative doses (Figs. 4, 5), may reflect a decrease in the blood supply to the ciliated epithelium in the maxillary sinus. Also the reduced effect of methacholine when given *i.a.* to animals pretreated with oxymetazoline might be explained by such changes in the circulation of the epithelium. This is supported by other investigations in the mucous membranes in the upper airways showing that α -adrenoceptor agonists when given *i.a.* decrease the blood flow (Malm, 1974) and when applied topically also cause microvascular injuries (Brånemark et al., 1975). However, the effect of α -adrenoceptor agonists on m.c. activity in disease was not investigated in the present study. The ciliated epithelium in the paranasal sinuses is situated between a capillary bed and a normally ventilated cavity. The energy supply to the epithelium and its m.c. activity is believed to be dependent both on the blood flow and the ambient air (Drettner & Aust, 1977; Reimer et al., 1981). In an occluded paranasal sinus, lined with a thick and impermeable secretion, the epithelium will be dependent of diffusion from the capillaries only. Thus a sympathomimetically induced reduction in the mucosal blood flow might retard the m.c. wave frequency comparatively more in disease than in health.

There seems to be no prior study showing the effect of α -adrenoceptor agonists on m.c. activity in the paranasal sinuses, but contradictory effects have been reported for other parts of the respiratory tract. In agreement with our results, α -adrenoceptor agonists have been shown to retard the m.c. transport rate in vitro in the rabbit trachea (Tsuchiya & Kensler, 1959) and in vivo in the nasal airways in human volunteers (Simon et al., 1977; Peerless & Noiman, 1980). On the other hand, Saketkhoo et al. (1978) reported that α -adrenoceptor agonists accelerated the m.c. transport rate of teflon discs (1 mm in diameter and 1.8 mg in weight) in the human nasal airways. The mechanism underlying this acceleration was unclear, but it cannot be excluded that the transport rate was influenced not only by the m.c. activity but also by improved nasal air flow induced by the locally administered α -adrenoceptor agonists. In addition, the disadvantage of using such large tracer discs has been stressed by Simon et al. (1977). In their study, performed in the same organ and with the aid of much smaller tracers (0.01–0.05 mm in diameter), α -adrenoceptor agonists were shown to retard the m.c. transport rate.

In our investigation, the decelerating effect of the agonists oxymetazoline and phenylephrine did not appear when they were given in cumulative doses (Figs. 4, 5). This may explain the previous reports that the α -adrenoceptor agonists norepinephrine (Kordik et al., 1952) and xylometazoline (Petruson, 1981) had no influence on m.c. activity, as in both these investigations the test substances were given in cumulative doses.

The adrenoceptor blocking substances phentolamine and propranolol did not influence the basal m.c. wave frequency in our study, which might indicate that during anesthesia there is no basal or reflex α or β -adrenergic activity present in the control of the m.c. wave frequency.

Only few other investigations have been undertaken to study the effect of adrenoceptor blocking substances on the m.c. activity. Ira-

vani (1972) and Iravani & Melville (1975) found that α and β -adrenoceptor blocking substances retarded the ciliary beat frequency in in vitro preparations of rat bronchial epithelium while we found no in vivo effect in rabbits. This discrepancy between results may be due to differences in preparation and recording techniques and in organs and species investigated. In studies of the mucus-secreting capacity of in vivo preparations of the feline trachea, Gallagher et al. (1975) found phentolamine to be without effect, whereas propranolol was found to produce a weak decrease in mucus output. Even though propranolol might influence the mucus-secreting apparatus also in the rabbit maxillary sinus, this alteration in the volume of the covering mucus sheet is too small to affect the m.c. wave frequency.

In conclusion the present investigation shows that sympathomimetic agonists with selectivity to different groups of adrenoceptors have opposite effects on m.c. wave frequency, i.e. acceleration by β_2 -adrenoceptor agonists and retardation by α_1 and α_2 -adrenoceptor agonists. This difference in effect between α and β -adrenoceptor agonists also illustrates the importance of using test substances with selectivity to different groups of adrenoceptors and not substances which act on both α and β -adrenoceptors, e.g. ephedrine and epinephrine.

Although it is well known that the results of animal experiments cannot always be transferred directly to clinical practice, it is worth considering that β_2 -adrenoceptor agonists, in addition to their established effect on pulmonary m.c. activity and airway mechanics, might be useful adjuncts to the m.c. defence system in the upper airways. Moreover, our finding that α -adrenoceptor agonists decrease the m.c. wave frequency might be of clinical interest, as it could indicate that airway decongestants acting on α -adrenoceptors play a role in the pathogenesis of airway diseases (cf. Feinberg & Feinberg, 1971; Peerless & Noiman, 1980).

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ZUSAMMENFASSUNG

Die Beeinflussung der mukoziliären Schlagfrequenz durch sympathomimetische Agonisten und Antagonisten wurde an der Kieferhöhlenschleimhaut von anesthetisierten Kaninchen studiert. Der nicht-selektive β -adrenorezeptoragonist Isoprenalin (0,005–10 $\mu\text{g/kg}$, i.a.) und der selektive β_2 -adrenorezeptoragonist Salbutamol (0,01–10 $\mu\text{g/kg}$, i.a.) zeigten eine dosisabhängige Erhöhung der mukoziliären Schlagfrequenz. Der β_1 -adrenorezeptoragonist Prenalterol (1–200 $\mu\text{g/kg}$, i.a.) zeigte keine Wirkung auf die basale mukoziliäre Aktivität. Der selektive α_1 -adrenorezeptoragonist Phenylephrin (0,01–20 $\mu\text{g/kg}$, i.a.) und der selektive α_2 -adrenorezeptoragonist Oxymetazolin (0,001–1 $\mu\text{g/kg}$, i.a.) zeigten beide eine dosisabhängige Erniedrigung der mukoziliären Schlagfrequenz. Propranolol, ein nicht-selektiver β -adrenorezeptorantagonist, in einer Dosis von 2–200 $\mu\text{g/kg}$, i.a., sowie 1 mg/kg, i.v., zeigte keinen Effekt auf die basale, ziliäre Aktivität. Ebenso auch der nicht-selektive α -adrenorezeptorantagonist Phentolamin (0,1–1 000 $\mu\text{g/kg}$, i.a.). Der Isoprenalin- und Salbutamoleffekt, beide in einer Dosisbreite von 0,01–10 $\mu\text{g/kg}$, i.a., wurde von Propranolol bei einer Dosis von 1–2 mg/kg, i.v., reduziert. In ähnlicher Weise verminderte Phentolamin (0,2 und 1 mg/kg, i.a.) die Wirkung von Oxymetazolin (0,01–10 $\mu\text{g/kg}$, i.a.). Aus den obigen Ergebnissen kann man erschliessen, dass die mukoziliäre Aktivität beim anesthetisierten Kaninchen unabhängig von der sympathischen Aktivität ist, und dass β_2 -adrenorezeptoragonisten beschleunigend, hingegen α_1 und α_2 -adrenorezeptoragonisten hemmend auf die mukoziliäre Schlagfrequenz einwirken.

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A PHARMACOLOGICAL EVALUATION OF THE SHORT-TERM EFFECT
OF CIGARETTE SMOKE ON MUCOCILIARY ACTIVITY

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ABSTRACT

The effect of whole cigarette smoke on mucociliary (m.c.) activity was recorded in the maxillary sinus in anesthetized rabbits. Puffs of cigarette smoke were delivered in a way that imitates the normal smoking habit in man. Cigarette smoke from nicotine cigarettes was found to accelerate the m.c. wave frequency in proportion to the nicotine content. Smoke from nicotine-free cigarettes and exposure to oxygen did not influence the m.c. activity. The stimulating effect of smoke drawn from nicotine cigarettes was inhibited by hexamethonium (0.2 mg/kg i.a.) and by atropine (0.2 mg/kg i.a.).

It is concluded that the accelerating effect on the m.c. activity of short-term exposure to cigarette smoke might be explained by stimulation of ganglion nicotinic receptors situated on parasympathetic post-ganglionic nerve cells.

INTRODUCTION

In epidemiological studies of airway diseases increased morbidity has been noted in smokers (Holland, 1968), but the mechanisms underlying the harmful effects of cigarette smoke on the mucous membranes have not been clarified (Wanner, 1977). As the mucociliary (m.c.) activity is believed to be one of the major defence mechanisms in the airways, a large number of investigations have been undertaken to study the effects of cigarette smoke on the m.c. transport and clearance rates and ciliary beat frequency.

However, the results from different investigations are conflicting. Short-term exposure to whole cigarette smoke has been shown (1) to cause ciliostasis and to retard m.c. transport rate in vivo and in vitro (e.g. Mendenhall & Shreeve, 1937; Battista & Kensler, 1970; Dalhamn, 1970; Isawa et al., 1980), (2) to accelerate m.c. clearance rate in vivo (Albert et al., 1969; Camner et al., 1971; Albert et al., 1975), (3) to have no substantial effects at all in vivo (Yeates et al., 1975). These discrepancies in reported data may to some extent be attributed to the use of different experimental approaches and to variations in the techniques for smoke exposure (Dalhamn & Rylander, 1969).

Attempts have also been made to clarify which component in the tobacco smoke is responsible for the observed effects on the m.c. activity. There are about 4,000 different compounds generated by burning tobacco (Jaffe, 1980). Some, like nicotine, have been claimed to cause ciliostasis and to abolish m.c. transport capacity (Carson et al., 1966; Guillerm et al., 1972; Isawa et al., 1980). As nicotine is absorbed from the mucous membranes in the upper and lower airways almost with the efficacy of i.v. administration (see Jaffe, 1980), short-term tobacco smoke effects on the m.c. activity could very well be mediated by this substance.

The major site of pharmacological action of nicotine on the peripheral nervous system is on the autonomic ganglia (see Taylor, 1980), but the effect is highly unpredictable as there are both stimulating and depressant phases of action. In small doses nicotine stimulates the ganglion cells directly and facilitates transmission of nerve impulses. When larger doses of the drug are applied, the initial stimulation is followed very quickly by a blockade of transmission (see Taylor, 1980). It is also difficult to predict the effect of nicotine on m.c. activity since ganglion nicotine receptors are situated both on parasympathetic and sympathetic postganglionic nerve cells, and because different parasympathomimetic and sympathomimetic agonists have been shown to have opposite effects on the m.c. wave frequency (Hybbinette & Mercke, 1981 b,c). The parasympathomimetic agonist methacholine and to a lesser extent also the sympathomimetic β -adrenoceptor agonists isoprenaline and salbutamol were all found to accelerate the m.c. wave frequency in a dose-related manner. On the other hand, the sympathomimetic α -adrenoceptor agonists phenylephrine and oxymetazoline were found to give a dose-dependent retardation of the wave frequency.

It is possible that some of the effects of tobacco smoke might be mediated via autonomous nerves, but there seems to be no prior study of tobacco effects on m.c. activity during blockade of neuroreceptor sites in the autonomous nervous system.

With these facts at hand it was considered to be of interest to determine whether any short-term effect on the m.c. activity of tobacco smoke could be explained in terms of increased or decreased activity in the autonomous nerves assumed to supply the mucous membrane in the rabbit maxillary sinus.

MATERIALS AND METHODS

The experiments were performed on 22 male, healthy and controlled rabbits weighing 1.7-2.5 kg from a white strain. Details of anesthetic and operative techniques were as described previously (Hybbinette & Mercke, 1981 a). The animals were anesthetized with urethane (2-2.5 g/kg) and the left facial or lingual artery was cannulated. The cannula was continuously perfused with physiological saline solution (5-10 ml/h) in order to keep it open and to compensate the animals for dehydration. This i.a. route was used for administering the receptor-blocking drugs. An opening of about 2x8 mm was made into the left maxillary sinus and the trepanation hole was immediately sealed with an antimist window and bone wax. Recordings of the m.c. activity were made from the fronto-superior part of the maxillary cavity with the aid of a photoelectric technique (Mercke et al., 1974). The m.c. wave pattern was monitored on an oscilloscope and recorded on an ink writer. Recordings of 10-20 sec duration were analyzed manually and with the aid of a computerized frequency calculator, and the frequency was expressed in waves/min. The induced frequency change was expressed as a percentage of the basal m.c. wave frequency immediately preceding the administration of the test substances. The wave frequency was recorded continuously during the stimulations and at intervals of 1-5 min otherwise.

ECG and rectal temperature were monitored continuously during the experiments and body temperature was adjusted to 37.0-38.5°C with the aid of a heating pad.

The following cigarettes were tested: (A) a king-size cigarette without filter; according to the manufacturer (British-American Tobacco Company Ltd, England) the smoke from 1 cigarette contained 1.8 mg nicotine, 17 mg CO and 29 mg tar; (B) a small cigarette with cellulose filter; the specification, according to the Swedish Tobacco Company, was 0.6 mg nicotine, 4 mg CO and 5 mg tar;

(C) a small aromatic herb cigarette, produced by Honey-rose Products Ltd, England, with cellulose filter; no tobacco or nicotine; CO and tar content not specified.

With the aid of a syringe 35 ml smoke was drawn during 2 sec once a minute from the cigarettes. This procedure allowed smoke to be drawn at most 13 times from a cigarette A and 8 times from a cigarette B or C. The cigarettes were smoked to a butt length of approximately 25 mm, excluding filters. Smoke was administered to the rabbits through a polyethylene catheter (inner diameter 1.5 mm) attached to the cranium holder and with its tip 2-3 mm in front of the nostril of the rabbit at the same side as the trepanized sinus.

For exposure a 3.5 ml puff of the smoke in the syringe was delivered during 3-4 consecutive inspiratory phases of respiration. The syringe was then cleared of the remaining smoke. This was repeated every minute. The animals were allowed to breathe fresh air between smoke puffs and during at least 20 min elapsing before exposure to another cigarette.

To test the effect of tobacco smoke during blockade of ganglion nicotinic receptors, hexamethonium bromide (Sigma Chemical Company) was used. Postganglionic parasympathetic receptors were blocked with the muscarine receptor antagonist atropine sulphate (ACO). These antagonists were delivered as standardized 3 sec, 0.2 ml retrograde injections through the cannula in the lingual or facial artery. Different test doses (dose/body weight) were given by varying the concentrations of the drug solutions. Injections of physiological saline at the same volume rates served as controls and did not influence the m.c. wave frequency.

For statistical evaluation Student's t-test for paired data was used. P-values smaller than 0.05 were considered significant.

RESULTS

Oxygen, when blown through the smoke catheter at a rate of 1 l/min for 1-5 min (6 rabbits) and exposure to smoke puffs delivered from nicotine-free cigarette C (6 rabbits) did not influence the basal m.c. wave frequency.

Smoke drawn from the nicotine-containing cigarettes A and B had a striking accelerating effect on the m.c. wave frequency, the responses appearing after a latency of 5-10 sec and remaining for 2-5 min in experiments using single 3.5 ml smoke puffs. Peak frequency values were obtained within 20-30 sec. Fig. 1 illustrates the accelerating effect on the m.c. wave frequency and wave pattern in one rabbit when exposed to 1 puff/min from cigarette A for 10 min.

The magnitude of the accelerating effect on the m.c. wave frequency and the duration of the response were dependent on whether the smoke was drawn from the higher nicotine-content cigarette A (1.8 mg/cig.) or from cigarette B (0.6 mg/cig.). The mean frequency increment, defined as the mean of all peak values in response to smoke drawn from one cigarette versus the basal prechallenge frequency value, was significantly larger for cigarette A ($56.0 \pm 17.0\%$ (mean $\pm$ S.D.); 19 rabbits) than for cigarette B ($35.9 \pm 12\%$; 8 rabbits; $p < 0.01$, by analysis of paired data in 6 rabbits). The difference in duration between the responses to smoke puffs drawn from cigarette A versus from cigarette B is also illustrated by Fig. 2 which shows that the m.c. wave frequency did not return to the basal prechallenge frequency level between smoke puffs drawn from cigarette A, whereas the wave frequency almost declined to the basal prestimulating value between smoke puffs from cigarette B. In addition Fig. 2 also illustrates the frequently observed phenomenon that the first 2-3 smoke puffs delivered from both cigarettes A and B induced a comparatively smaller in-

crease of the m.c. wave frequency than did the following smoke puffs.

The responses to cigarette smoke were reproducible, which was demonstrated in experiments on 9 rabbits exposed to repeated series of smoke puffs drawn from 3-7 cigarettes smoked at time intervals of 20-60 min. One example is illustrated in Fig. 3, showing the accelerating effect on the m.c. wave frequency in one rabbit when exposed to series of smoke puffs drawn from 7 cigarettes (5 cig. A and 2 cig. B).

Hexamethonium, a reversible blocker of nicotine receptors in autonomic ganglia, did not per se influence the basal m.c. wave frequency when given in the dose range 0.00001-5 mg/kg (7 rabbits). Hexamethonium (0.1-5 mg/kg) given 1-2 min in advance of exposure to series of smoke puffs delivered from cigarette A reduced or abolished the responses to the first 3-4 smoke puffs (5 rabbits). As the first 2-3 smoke puffs delivered from both cigarette A and B induced a comparatively smaller increase than did the following smoke puffs, also in animals not given hexamethonium (see Fig. 2) additional experiments were performed (6 rabbits) in which hexamethonium (0.2 mg/kg) was given 30 sec after the third or fourth puff during periods of exposure to smoke drawn from cigarette A. The results from these experiments showed that hexamethonium not only blocked the responses to cigarette smoke for 2-5 min, but also that the increase in wave frequency caused by the first 3-4 puffs was reduced to the basal prestimulating frequency level within 20-60 sec. One example is given in Fig. 4, which illustrates the effect of smoke from cigarette A before and after an injection of hexamethonium (0.2 mg/kg i.a.).

Atropine, a blocker of postganglionic muscarine receptors, was given in a dose of 0.2 mg/kg i.a. both in advance and during periods of smoking. When the atropine blockade preceded by 2 min exposure to smoke from ciga-

rette A (6 rabbits), the smoke effect on the m.c. wave frequency was either markedly reduced as compared to the previously found and expected effect or even abolished. The duration of this blockade varied between different animals studied. In 3 out of the 6 rabbits the blocking effect lasted only during the first 3-4 puffs, whereas in the remaining 3 animals it was observed during the whole period of exposure to a cigarette. The percentage increase in m.c. wave frequency, as calculated from the peak values following the first 4 puffs, was significantly reduced by atropine 0.2 mg/kg ($p < 0.01$, by analysis of paired data; 6 rabbits). When atropine 0.2 mg/kg was given during periods of exposure to smoke from cigarette A (4 rabbits) the effect of the smoke after atropine was markedly reduced or abolished. In different animals the latency until maximum blocking effect was observed varied between 1.5 and 4 min. The duration of this blockade also varied between different animals and was approximately 5-10 min, except in one rabbit, in which the effect of tobacco smoke on the m.c. wave frequency was inhibited for 30 min after the administration of atropine. Figure 5 shows the effect of smoke from cigarette A on the m.c. wave frequency in one rabbit before and after an injection of atropine (0.2 mg/kg i.a.) given 30 sec after the fifth puff.

DISCUSSION

The present investigation indicates that exposure to cigarette smoke, when delivered in a way that imitates the smoking pattern in man, accelerates the m.c. wave frequency. There is a positive correlation between the nicotine content in the smoke delivered and the magnitude of the acceleration. This effect is weakened or inhibited by the receptor antagonists hexamethonium and atropine.

Various experimental factors influencing the evaluation of the effect of tobacco smoke on the m.c. activity (such as mode of administration, duration and volume of smoke exposure, recording technique, organ and species studied and in vitro or in vivo observations) have been analyzed by Dalhamn & Rylander (1969). The dose of tobacco constituents available to a smoker depends not only on the cigarette specification but also on the way in which the cigarettes are smoked (Guillerm & Radziszewski, 1978; Rawbone et al., 1978). In the present investigation the animals were exposed to cigarette smoke in a way that imitates the normal smoking habit in man. In humans, an average of about 35 ml of smoke is inhaled during 2 sec once every minute (see Corresta Standards in Neurath, 1968). The reduction factor for the smoke volume in animal experiments as compared with humans was analyzed by Dalhamn & Rylander (1969) and the puff volume of 3.5 ml used in the present study follows their recommendation. In earlier in vivo and in vitro investigations in the trachea of rabbits and cats this puff volume was found to be sufficiently large to cause arrest of the mucus transport and ciliostasis (Dalhamn, 1970).

In the present study the animals breathed through their normal respiratory channels. The target organ, the sinus mucous membrane, was only minimally exposed to environmental disturbances such as variations in humidity and temperature in the environmental air, factors

known to influence the m.c. activity (Mercke & Tore-malm, 1976). Although the inspired air was contaminated with warm and dry cigarette smoke for periods of exposure, the volume of the puffs was small in comparison with the total volume of inspired air (Dalhamn & Rylander, 1969). As the gas exchange between the maxillary sinus and the nasal airways is believed to be relatively small (c.f. Drettner & Aust, 1977) variations in physical factors in the ambient air are not expected to have biased the present results. This is also supported by the absence of influence on the sinus m.c. activity in experiments with nicotine-free cigarettes and pure oxygen. Nor was any other adverse effect of cigarette smoke such as ciliostasis noted, even in experiments where the animals were exposed to repeated series of puffs for 4 hours (Fig. 3).

The photoelectric, non-invasive recording technique (Mercke et al., 1974) is advantageous because continuous recording of the m.c. activity is possible and rapid changes in the m.c. activity can be detected. A smoke-induced increase in m.c. wave frequency with a brief latency (5-10 sec) and a short duration (2-5 min) can be detected with the present recording technique whereas it might be overlooked in experiments where the effect of tobacco smoke on the m.c. activity is measured as changes in m.c. transport rate only several minutes after exposure to cigarette smoke (e.g. Isawa et al., 1980).

In the present investigation smoke drawn from cigarette A (nicotine content 1.8 mg/cig.) had a significantly greater potency to accelerate the m.c. wave frequency than smoke from cigarette B (nicotine content 0.6 mg/cig.). The smoke from the nicotine-free cigarette C did not influence the basal m.c. frequency. Although several tobacco smoke constituents may influence the m.c. activity (Guillerm et al., 1972), there is a positive correlation between the nicotine content in the smoke puffs and the magnitude of the acceleration of

the m.c. frequency.

The increase in m.c. wave frequency might be explained by stimulation of ganglion nicotinic receptors since hexamethonium blocked the effect of the smoke (Fig. 4). Although ganglion nicotinic receptors are situated both on sympathetic and parasympathetic postganglionic nerve cells, the tobacco effect on the m.c. wave frequency was probably mediated mainly by postganglionic parasympathetic nerve fibres since atropine almost completely reduced the effect of tobacco smoke (Fig. 5). Stimulation of nicotinic receptors on sympathetic nerve cells cannot be excluded. However, as such stimulation results in a release of norepinephrine, which mainly acts on α_1 , α_2 and β_1 -adrenoceptors (Wikberg, 1979), sympathetic nerve stimulation is very unlikely to accelerate the m.c. wave frequency; agonists acting on these adrenoceptors have been shown to either decrease (α_1 and α_2) or to have no effect at all (β_1) on the m.c. wave frequency (Hybbinette & Mercke, 1981 c).

Tobacco smoke has been found to increase in vivo bronchial clearance rate in man (Camner et al., 1971; Albert et al., 1975) and in the donkey (Albert et al., 1969), which supports the present results. Although these investigations indicate that tobacco smoke accelerates the m.c. activity also in other organs than that studied in the present investigation, they do not offer any evidence regarding the possible neuronal mechanisms underlying the effect of tobacco smoke on m.c. activity.

In contrast to our finding, tobacco smoke has been shown in several in vitro investigations to arrest ciliary beating and m.c. transport (e.g. Mendenhall & Shreeve, 1937; Dalhamn, 1970). However, these investigations do not necessarily conflict with the present results because the accelerating effect of nicotine on m.c. activity might not appear in in vitro airway preparations that are denervated and deprived of blood circulation.

Dalhamn (1970) reported cigarette smoke to cause cilio-stasis and to arrest mucus transport both in vivo and in vitro in the trachea of the cat and the rabbit. But as these in vivo experiments were performed in transected tracheal preparations, the reason for no accelerating tobacco effect appearing might well be denervation if efferent postganglionic vagal nerve fibres were cut.

In a recent in vivo investigation in dogs Isawa and collaborators (1980) reported cigarette smoke to cause a nicotine dose-dependent decrease in pulmonary m.c. transport rate. The dogs were exposed to fairly large smoke and nicotine doses. Tobacco smoke has also been reported to retard m.c. activity in vivo in chicken (Battista & Kensler, 1970) with smoke delivered in accordance with smoking habits in man. This finding might be explained by dehydration of the investigated chicken tracheal mucous membrane and its mucus sheet because fresh air and smoke from nicotine-free cigarettes also retarded the m.c. transport rate.

In conclusion the present investigation shows that cigarette smoke when given in a way that imitates the normal smoking habit in man accelerates the m.c. wave frequency and that this acceleration can be attributed to stimulation of ganglion nicotinic receptors situated on postganglionic parasympathetic nerve cells.

ZUSAMMENFASSUNG

Die Wirkung von Zigarettenrauch auf die mukoziliäre Aktivität wurde an Kieferhöhlen anesthesierter Kaninchen untersucht. Es wurden Zigarettenrauchschübe verabreicht, die den Gewohnheiten des Rauchens beim Menschen entsprechen. Nikotinhaltiger Zigarettenrauch bewirkte eine von der Nikotinkonzentration abhängige Erhöhung der mukoziliären Schlagfrequenz. Der Rauch nikotinfreier Zigaretten und ebenso die Applikation reinen Sauerstoffs zeigte keine Wirkung auf die mukoziliäre Aktivität. Die stimulierende Wirkung nikotinhaltigen Zigarettenrauches wurde durch Hexamethonium (0,2 mg/kg i.a.) und durch Atropin (0,2 mg/kg i.a.) aufgehoben.

Die stimulierende Wirkung in Form einer kurzzeitigen Erhöhung der mukoziliären Schlagfrequenz kann durch Erregung ganglionärer, auf Nikotin ansprechender Rezeptoren an parasympathischen postganglionären Nervenzellen erklärt werden.

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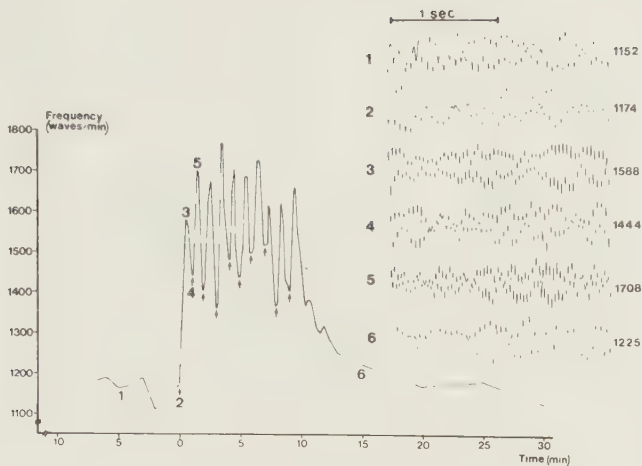


Fig. 1. The effect on the m.c. wave frequency in one rabbit exposed to a series of smoke puffs from cigarette A is shown to the left. Arrows indicate each puff. Examples of the original wave recordings from 6 instants during the experiment (large figures) are given to the right with the respective calculated wave frequencies in waves/min.



Fig. 2. The effect on the m.c. wave frequency in one rabbit exposed to series of smoke puffs from cigarette A and from cigarette B. Arrows indicate each puff, and markers on the abscissa 1 min intervals.

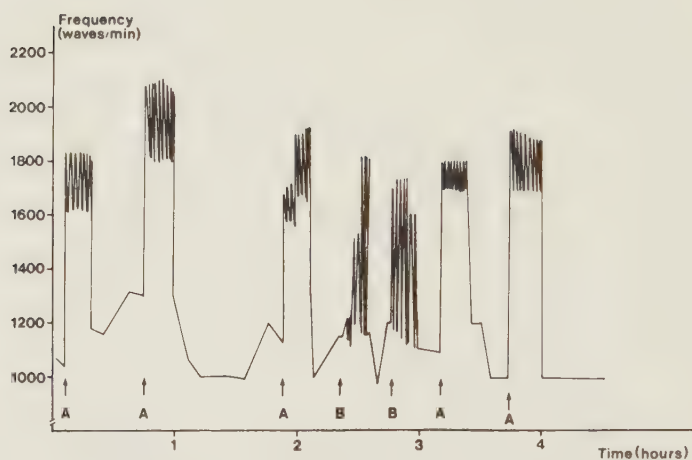


Fig. 3. The effect on the m.c. wave frequency in one rabbit exposed to series of smoke puffs from 7 cigarettes (5 cigarettes A and 2 cigarettes B). Arrows indicate the first puff drawn from each cigarette.

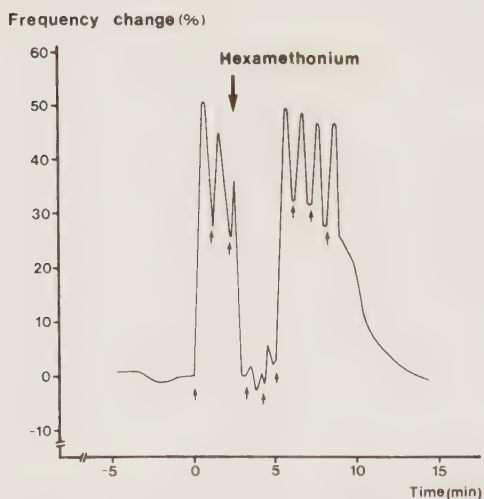


Fig. 4. The effect of hexamethonium 0.2 mg/kg i.a. on the m.c. wave frequency in one rabbit exposed to a series of smoke puffs from cigarette A. Arrows under curve indicate each puff. The ordinate represents frequency change given as a percentage of the wave frequency at the instant the smoke exposure began. Basal frequency zero level is 1236 waves/min.

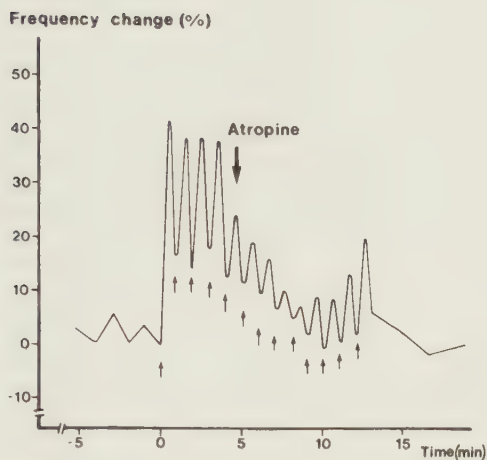


Fig. 5. The effect of atropine 0.2 mg/kg i.a. on the m.c. wave frequency in one rabbit exposed to a series of smoke puffs from cigarette A. Arrows under curve indicate each puff. The ordinate is as in Fig. 4, and the basal frequency zero level is 1356 waves/min.

